Introduction

The first part of this chapter covers blood group terminology and how antigens are organized into a classification scheme. Conventions for the correct use of terminology are included so that the student will be able to accurately communicate information about antigens and antibodies, **phenotypes**, and **genotypes**.

The ABO and Rh blood groups are the most significant in transfusion practice. However, there are over 300 RBC antigens that are formally recognized internationally. Blood group antigens are defined by carbohydrates (sugars) attached to glycoprotein or glycolipid structures or by amino acids on a protein. The carbohydrate blood groups Lewis, P, and I will be presented first. The antigens of these carbohydrate systems (like ABO and H) are not encoded by their genes directly. Rather, the genes encode specific **glycosyltransferases** that in turn synthesize the carbohydrate epitopes by sequential addition of sugars to a precursor.

The next blood groups discussed are MNS, Duffy, Kell, and Kidd. These are significant in routine transfusion medicine; antibodies to these antigens are more commonly encountered. The chapter then addresses the remainder of the blood groups and selected antigens. Antibodies to these latter will be rarely encountered, but it is important for clinical laboratorians to know that these other antigens and corresponding antibodies exist. While there is more information presented here than is required to work capably at the bench, students will be better prepared for those real-life situations when they encounter one of these uncommon antibodies.

Antigen and antibody characteristics are discussed for each blood group presented. Included are the effects of enzymes and chemical treatments on test RBCs. This information is a tool for antibody identification. For example, if an antibody no longer reacts with a panel of RBCs after the RBCs have been pretreated with an enzyme such as ficin or papain, then only those antibodies that don't react after enzyme treatment are considered for the specificity, and all other specificities can be excluded. These antigen and antibody characteristics are summarized on the front and back cover of the book so that students can easily access pertinent information while performing routine antibody identification.

Blood Groups and Terminology

A blood group system is one or more antigens produced by **alleles** at a single gene **locus** or at loci so closely linked that crossing over does not occur or is very rare.[1] With a few notable exceptions, most blood group genes are located on the autosomal chromosomes and demonstrate straightforward Mendelian inheritance.

Most blood group alleles are **codominant** and express a corresponding antigen. For example, a person who inherits alleles *K* and *k* expresses both K and k antigens on his or her RBCs. Some genes code for complex structures that carry more than one antigen (e.g., the glycophorin B structure, which carries S or s antigen, also carries the U antigen).

Silent, or **amorphic**, alleles exist that make no antigen, but they are rare. When paired chromosomes carry the same silent allele, a **null phenotype** results. Null phenotype RBCs can be very helpful when evaluating antibodies to unknown **high-prevalence** antigens. For example, an antibody reacting with all test cells except those with the phenotype Lu(a–b–) may be directed against an antigen in the Lutheran system or an antigen phenotypically related to the Lutheran system. In some blood group systems, the null phenotype results in RBC abnormalities.

Some blood group systems have regulator or modifying genes, which alter antigen expression. These are not necessarily located at the same locus as the blood group genes they affect and may segregate independently. For example, RBCs with the dominant type of Lu(a–b–) have suppressed expression of all the antigens in the Lutheran blood group system as well as many other antigens, including P1 and i. This modifying gene is inherited independently of the genes coding for Lutheran, P1, and i antigens.

Blood group antigens are detected by alloantibodies, which occur naturally (i.e., without a known immune stimulus) or as a response by the immune system after exposure to non-self RBC antigens introduced by blood transfusion or pregnancy. Over the last century, blood group antigens have been named using several styles of symbols: uppercase single letters, uppercase and lowercase letters to represent alleles, letters derived from the name of the system (some with superscripts to represent alleles), letter symbols followed by numbers, and finally the more recent use of all uppercase letters.

Although gene and antigen names seem confusing at first, certain conventions are followed when writing alleles, antigens, and phenotypes.[1] Some examples are given in Table 8–1. Genes are written in italics or underlined when italics are not available (e.g., when handwritten), and their allele number or letter is always superscript. Antigen names are written in regular type without italics or underlining; some antigens have numbers or superscript letters. A phenotype is a description of which antigens are present on an individual's RBCs and simply indicates the results of serologic tests on those RBCs. It is important to use subscripts, superscripts, and italics appropriately. For example, A1, A_1 and A^1 mean the antigen, the phenotype, and the allele respectively.

How the phenotype is written depends on the antigen nomenclature and whether letters or numbers are used. For letter antigens, a plus sign or minus sign written on the same line as the antigen is used to designate that the antigen is present or absent, respectively. Examples are M+ and K–. For antigens that have superscripts, the letter of the superscript is placed in parentheses on the same line as the letter defining the antigen—for example, Fy(a+) and Jk(a–). When testing for both of the antithetical antigens, both results are written within the parentheses—for instance, Fy(a–b+). For antigens that have a numerical designation, the letter(s) defining the system is followed by a colon, followed by the number representing the antigen. No plus sign is written if the antigen is present, but a minus sign is placed before the

Table 8–1 Examples of Terminology for Genes, Antigens, Antibodies, and Phenotypes

	GENE		ANTIGEN	ANTIBODY	PHENOTYPE	
System	**Conventional**	**ISBT**			**Antigen Positive**	**Antigen Negative**
Lewis	_Le_ _le_	_LE_	Le^a Le^b	Anti-Le^a Anti-Le^b	Le(a+) Le(b+)	Le(a–) Le(b–)
MNS	_M_ _N_ _S_ _s_	_MNS*1_ _MNS*2_ _MNS*3_ _MNS*4_	M N S s	Anti-M Anti-N Anti-S Anti-s	M+ N+ S+ s+	M– N– S– s–
Kell	_K_ _k_	_KEL*1_ _KEL*2_	K k	Anti-K Anti-k	K+ k+	K– k–
Kidd	_Jk_^{a} _Jk_^{b}	_JK*1_ _JK*2_	Jk^a Jk^b	Anti-Jk^a Anti-Jk^b	Jk(a+) Jk(b+)	Jk(a–) Jk(b–)

negative result, and multiple results are separated by a comma (e.g., Sc:–1,2). Phenotypes of more than one blood group system are separated by a semicolon—for example, S+s+; K–; Fy(a+b–).

One must remember that serologic tests determine only RBC phenotype, not genotype. A genotype is composed of the actual genes that an individual has inherited and can be determined only by family or DNA studies. Sometimes the genotype can be predicted or inferred by the phenotype. When based on results from RBC antigen typing, the genotype is a *probable interpretation* as to which genes the individual carries in order to have the observed phenotype.

Antibodies are described by their antigen notation with the prefix *anti-*, including a hyphen before the antigen symbol. Use of correct blood group terminology, especially for antibodies identified in a patient's serum, is very important so that correct information is conveyed for patient care. Some examples of correct and incorrect terminology are given in Table 8–2.

Numeric terminology was originally introduced for the Kell and Rh systems and was subsequently applied to other systems. To facilitate computer storage and retrieval of blood group information and to help standardize blood group system and antigen names, the International Society of Blood Transfusion (ISBT) formed the Working Party on Terminology for Red Cell Surface Antigens in 1980.[2] The numeric system this group proposed was not intended to replace traditional terminology but rather to enable communication on computer systems where numbers are necessary. Each antigen is given a six-digit identification number. The first three digits represent the system, collection, or series, and the second three digits identify the antigen. The antigens within the system are numbered sequentially in order of discovery. Each system also has an alphabetical symbol. For example, using the ISBT terminology, the K antigen is 006001 with the first three numbers (006) representing the system and the second three numbers (001) representing the number assigned to K. Antigens can also be written using the system symbol followed by the antigen number; for instance, KEL1 (the redundant sinistral zeroes can be omitted). This committee's work is ongoing, and the assignment of RBC antigens to blood group systems is periodically updated. The first monograph was released in 1990, and updates are published in Vox Sanguinis[3] and on the Working Party's page of the ISBT website (www.isbtweb.org).

Table 8–2 Examples of Correct and Incorrect Terminology

CORRECT	INCORRECT
Fy(a+)	Fy^a+, Fy$^{(a+)}$, Fya+, Fya(+), Duffy a-positive, Duffy^a+
Fy(a–b+)	Fy^{a-b+}, Fy^a(–)Fy^b(+)
Anti-Fy^a	Anti Fy^a, anti-Duffy, anti-Duffy^a
K	Kell (name of system), K1
Anti-k	Anti-Cellano, anti-K2
M+N–	M(+), MM
Ge:–2	Ge2–, Ge:2–, Ge2-negative

The ISBT assigns RBC antigens to a system, collection, or low- or high-prevalence series. This chapter uses traditional terminology, but the ISBT symbol and number are indicated for the blood group systems and collections discussed.

As defined by the ISBT, a blood group system "consists of one or more antigens controlled at a single gene locus, or by two or more very closely linked homologous genes with little or no observable recombination between them."[4] Each system is genetically distinct. To date, there are 30 blood group systems (Table 8–3).[3]

Collections are antigens that have a biochemical, serologic, or genetic relationship but do not meet the criteria for a system.[4] Antigens classified as a collection are assigned a 200 number. Some of the previously established collections have been made obsolete as criteria have been met to establish a system or incorporate antigens into an existing system. See Table 8–4 for a listing of the ISBT collections.

All remaining RBC antigens that are not associated with a system or a collection are catalogued into the 700 series of

Table 8–3 International Society of Blood Transfusion (ISBT) Blood Group Systems

NUMBER	NAME	SYMBOL	GENE NAME ISBT (HGNC)	CHROMOSOMAL LOCATION	NO. OF AGS
001	ABO	ABO	*ABO (ABO)*	9q	4
002	MNS	MNS	*MNS (GYPA, GYPB, GYPE)*	4q	46
003	P1PK	P1PK	*P1PK (A4GALT)*	22q	2
004	Rh	RH	*RH (RHD, RHCE)*	1p	52
005	Lutheran	LU	*LU (LU)*	19q	20
006	Kell	KEL	*KEL (KEL)*	7q	32
007	Lewis	LE	*LE (FUT3)*	19p	6
008	Duffy	FY	*FY (DARC)*	1q	5
009	Kidd	JK	*JK (SLC14A1)*	18q	3
010	Diego	DI	*DI (SLC4A1)*	17q	22
011	Yt	YT	*YT (ACHE)*	7q	2
012	Xg	XG	*XG (XG)* *MIC2 (CD99)*	X	2
013	Scianna	SC	*SC(ERMAP)*	1p	7
014	Dombrock	DO	*DO (ART4)*	12p	7
015	Colton	CO	*CO (AQP1)*	7p	4
016	Landsteiner-Wiener	LW	*LW (ICAM4)*	19p	3
017	Chido/Rodgers	CH/RG	*CH/RG (C4A, C4B)*	6p	9
018	H	H	*H (FUT1)*	19q	1
019	Kx	XK	*XK (XK)*	Xp	1
020	Gerbich	GE	*GE (GYPC)*	2q	11
021	Cromer	CROM	*CROM (CD55)*	1q	16
022	Knops	KN	*KN (CR1)*	1q	9
023	Indian	IN	*IN (CD44)*	11p	4
024	Ok	OK	*OK (BSG)*	19p	3
025	Raph	RAPH	*RAPH (CD151)*	11p	1
026	John Milton Hagen	JMH	*JMH (SEMA7A)*	15q	6
027	I	I	*IGNT (GCNT2)*	6p	1
028	Globoside	GLOB	*GLOB (B3GALT3)*	3q	1
029	Gill	GIL	*GIL (AQP3)*	9p	1
030	Rh-associated glycoprotein	RHAG	*RHAG (RHAG)*	6p	2

HGNC = Human Gene Nomenclature Committee

Table 8–4 International Society of Blood Transfusion Blood Group Collections

COLLECTION			ANTIGEN	
Number	**Name**	**Symbol**	**Number**	**Symbol**
205	Cost	COST	205001 205002	Cs^{a} Cs^{b}
207	Ii	I	207002	i
208	Er	ER	208001 208002 208003	Er^{a} Er^{b} Er3
209		GLOB	209003 209004	LKE PX2
210			210001 210002	Le^{c} Le^{d}
212	Vel	VEL	212001 212002	Vel ABTI
213		MN CHO	213001 213002 213003 213004 213005 213006	Hu M$_{1}$ Tm Can Sext Sj

Antigens in shaded boxes are discussed in text.

low-prevalence antigens or the 901 series of high-prevalence antigens. Refer to Table 8–5 for a listing of low- and high-prevalence antigens recognized by the ISBT. Antigens in these series represent those with a prevalence of less than 1% or more than 90% of most random populations, respectively. As terminology has evolved, the terms *high-* and *low-incidence*, also previously known as *high-* and *low-frequency*, are currently being replaced by the terms *high-* and *low-prevalence*, reflecting the occurrence of an inherited characteristic at the phenotypic level.[5]

The Lewis (007) System

The Lewis blood group system is unique because the Lewis antigens are not intrinsic to RBCs but are on type 1 glycosphingolipids that are passively adsorbed onto the RBC membrane from the plasma. The Lewis system was named after one of the first individuals to make the antibody, reported by Mourant in 1946.[6] This antibody, later called anti-Le^{a}, agglutinated RBCs from about 25% of English people.[7] In 1948, an antibody, later called anti-Le^{b}, was found that reacted with Le(a–) individuals. It was thought that Le^{a} and Le^{b} were antithetical antigens, but we now know this is not so because they do not result from alternative alleles of a single gene. Rather, they result from the interaction of two fucosyltransferases encoded by independent genes, *Le* and *Se*.

The Lewis blood group system has been assigned the ISBT system number 007 and the system symbol LE.

Table 8–5 International Society of Blood Transfusion Antigens of Low (700 Series) and High (901 Series) Prevalence

	NUMBER	NAME	SYMBOL	NUMBER	NAME	SYMBOL
Lows	700002	Batty	By	700039	Milne	
	700003	Christiansen	Chr^{a}	700040	Rasmussen	RASM
	700005	Biles	Bi	700044		JFV
	700006	Box	Bx^{a}	700045	Katagiri	Kg
	700017	Torkildsen	To^{a}	700047	Jones	JONES
	700018	Peters	Pt^{a}	700049		HJK
	700019	Reid	Re^{a}	700050		HOFM
	700021	Jensen	Je^{a}	700052		SARA
	700028	Livesay	Li^{a}	700054		REIT
Highs	901002	Langereis	Lan	901009	Anton	AnWj
	901003	August	At^{a}	901012	Sid	Sd^{a}
	901005		Jr^{a}	901014		PEL
	901008		Emm	901016		MAM

Antigens in shaded boxes are discussed in text.

Basic Concepts

There are several Lewis antigens, but the two of primary concern are Le^a and Le^b. The Lewis (*Le, FUT3*) gene is located on chromosome 19 (at 19p13.3), as is the **secretor** (*Se, FUT2*) gene at 19q13.3. There are two alleles at the Lewis locus, *Le* and the amorph *le*, and there are two alleles at the secretor locus, *Se* and the amorph *se*. The biochemistry and interaction of the genes at these two loci will be explained in a later section, but in short, the *Le* gene must be present for a precursor substance to be converted to Le^a, but the *Se* gene must also be present for conversion to Le^b. The four phenotypes resulting from the interaction of these two genes are shown in Table 8–6.

In 1948, it was observed that individuals with Le(a+) RBCs were mostly nonsecretors of ABH.[8] As a result, in general for adults, Le(a+b–) RBCs are from ABH nonsecretors and Le(a–b+) RBCs are from ABH secretors (refer to Chapter 6, "The ABO Blood Group System"). Individuals with the Le(a–b–) RBC phenotype are either secretors or nonsecretors.[7] The Le(a–b–) phenotype is found more frequently among Africans. The Le(a+b+) phenotype is rare among whites and Africans but is more frequent among Asians, with a prevalence of 10% to 40%.[9] The antigens of the Lewis blood group system recognized by the ISBT are listed in Table 8–7.

Lewis antigens are not expressed on cord RBCs and are often diminished on the mother's RBCs during pregnancy. Lewis antigens are found on lymphocytes and platelets and on other tissues such as the pancreas, stomach, intestine, skeletal muscle, renal cortex, and adrenal glands.[9] In addition, soluble Lewis antigens are found in saliva as glycoproteins.

Lewis antigens are resistant to treatment with the enzymes ficin and papain, dithiothreitol (DTT), and glycine-acid EDTA. Reactivity of Lewis antibodies can be greatly enhanced by testing with enzyme-treated RBCs; hemolysis of enzyme-treated RBCs may be seen if serum is tested.

Lewis Antibodies

Lewis antibodies are often naturally occurring and made by Le(a–b–) persons; that is, they occur without any known RBC stimulus. They are generally IgM and do not cross the placenta. Because of this and because the Lewis antigens are not well developed on fetal RBCs, the antibodies do not cause hemolytic disease of the fetus and newborn (HDFN). Anti-Le^a and anti-Le^b may occur together and can be neutralized by the Lewis substances present in plasma or saliva or with commercially prepared Lewis substance. Lewis antibodies occur quite frequently in the sera of pregnant women who transiently exhibit the Le(a–b–) phenotype.

Table 8–6 Phenotypes of the Lewis System

PHENOTYPE	ADULT PHENOTYPE PREVALENCE (%)	
	Whites	Blacks
Le(a+b–)	22	23
Le(a–b+)	72	55
Le(a–b–)	6	22
Le(a+b+)	Rare	Rare

Table 8–7 Antigens of the Lewis Blood Group System

ANTIGEN	ISBT NUMBER	COMMENT
Le^a	LE1	
Le^b	LE2	
Le^{ab}	LE3	Anti-Le^{ab} reacts with Le(a+b–) and Le(a–b+) RBCs from adults and with 90% cord RBCs.*
Le^{bH}	LE4	Anti-Le^{bH} reacts with group O Le(b+) and A_2 Le(b+) RBCs.
ALe^b	LE5	Anti-Ale^b reacts with group A_1 Le(b+) and A_1B Le(b+) RBCs.
BLe^b	LE6	Anti-BLe^b reacts with group B Le(b+) and A_1B Le(b+) RBCs.

*Reactive cord RBCs [serologically Le(a–b–)] are from babies with *Le* gene.

Most Lewis antibodies agglutinate saline suspended RBCs, but these agglutinates are often fragile and can be easily dispersed if the cell button is not gently resuspended after centrifugation. Lewis antibodies can bind complement, and when fresh serum is tested, anti-Le^a may cause in vitro hemolysis of incompatible RBCs, though this is more often seen with enzyme-treated RBCs than with untreated RBCs.

Anti-Le^a is the most commonly encountered of the Lewis antibodies and is often detected in room temperature tests, but it sometimes reacts at 37°C and in the indirect antiglobulin test. Rare hemolytic transfusion reactions (HTR) have been reported in patients with anti-Le^a who were transfused with Le(a+) RBCs, so anti-Le^a that are reactive at 37°C, particularly those that cause in vitro hemolysis, should not be ignored.[1] It is relatively easy to find Le(a–) units since 80% of the population are secretors. Obtaining donor units that are typed Le(a–) with reagent antisera is generally not considered necessary for these patients; units that are crossmatch compatible in tests performed at 37°C are acceptable. Persons whose RBCs are Le(a–b+) do not make anti-Le^a, because small amounts of unconverted Le^a are present in their plasma and saliva.

Anti-Le^b is not as common or generally as strong as anti-Le^a. It is usually an IgM agglutinin and can bind complement. Anti-Le^b is infrequently made by Le(a+b–) individuals and can be classified into two categories: anti-Le^{bH} and anti-Le^{bL}. Anti-Le^{bH} reacts best when both the Le^b and the H antigens are present on the RBC, such as group O and A_2 cells. Anti-Le^{bH} represents an antibody to a compound antigen. Anti-Le^{bL} recognizes any Le^{b} antigen regardless of the ABO type. Anti-Le^{bH} should be suspected when anti-Le^{b} is identified with a panel of RBCs (group O) but most or all group A donor units are crossmatch compatible (as only about 20% of the group A RBC units will be from group A$_2$ individuals).

Lewis antigens are not intrinsic to the RBC membrane and are readily shed from transfused RBCs within a few days of transfusion. Also, Lewis blood group substance present in transfused plasma neutralizes Lewis antibodies in the recipient.[5] This is why it is exceedingly rare for anti-Le^{a} or anti-Le^{b} to cause hemolysis of transfused RBCs.

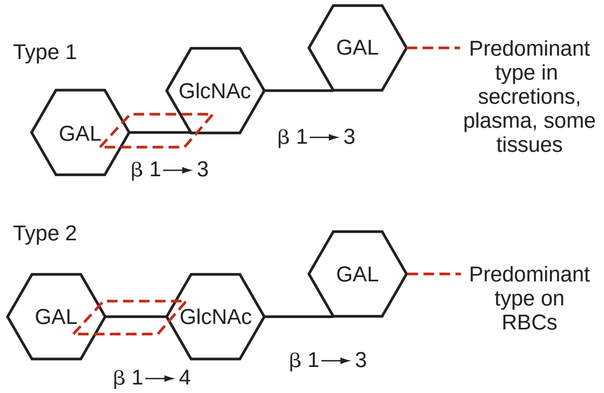

Figure 8–1. Precursor type 1 and type 2 chains. The type 1 chain has a beta 1→3 linkage of the terminal galactose (Gal) to the *N*-acetylglucosamine (GlcNAc) of the precursor structure. The type 2 chain has a beta 1→4 linkage of the terminal galactose.

Genetics and Biosynthesis

Advanced Concepts

The Lewis gene (*FUT3*) is linked to *Se* (*FUT2*) and *H* (*FUT1*), all located on chromosome 19. The synthesis of Lewis antigens depends on the interaction of the **transferases** produced by the Lewis and secretor genes. The Lewis and secretor transferases preferentially fucosylate type 1 chains, whereas the *H* gene (*FUT1*) preferentially fucosylates type 2 chains. Type 1 chain refers to the beta linkage of the number 1 carbon of galactose to the number 3 carbon of *N*-acetylglucosamine (GlcNAc) residue of the precursor structure (Fig. 8–1). As shown in Figure 8–2, secretor α1,2-L-fucosyltransferase adds a terminal fucose to the type 1 chain to form type 1 H. The *Le* allele codes for α1,4-L-fucosyltransferase, which transfers L-fucose to type 1H chain on glycoprotein or glycolipid structures to form Le^{b}. Small amounts of Le^{a} are made before the secretor enzyme is able to add the terminal fucose. If these individuals also have *A* or *B* genes, type 1H structures will be converted to A or B structures and the *Le* fucosyltransferase will then produce ALe^{b} or BLe^{b} (see **Fig. 8–2**).

Individuals who are nonsecretors do not have the enzyme to convert type 1 chains in secretions to type 1H. Consequently, the type 1 precursor is available for action by the *Le* fucosyltransferase, with the result of Le^{a} antigen in secretions and Le(a+b–) RBCs. The Le^{a} antigen cannot be converted to Le^{b} because of steric hindrance from the fucose added to make Le^{a}. Individuals with a weak *Se* gene, common in Asia, produce a fucosyltransferase that competes less effectively with the *Le* fucosyltransferase, resulting in RBCs with the Le(a+b+) phenotype.[7] The Le(a+b+) phenotype is rare in European populations, and it occurs in 10% to 40% of some Asian populations.[9] Mutations that result in inactivation of the fucosyltransferase, represented by the amorphic or silent *le* allele, are responsible for the Le(a–b–) phenotype.

All Le(a–b+) individuals are ABH secretors and also secrete Le^{a} and Le^{b}; very little Le^{a} will be detected in the plasma or on the RBCs. All Le(a+b–) individuals are ABH nonsecretors, yet all secrete Le^{a}. Approximately 78% to 80% of whites are secretors, and 20% are nonsecretors. In terms of Le(a–b–) individuals, 80% are ABH secretors, and 20% are ABH nonsecretors. The interaction of Lewis, secretor, and ABO genes is summarized in Table 8–8.

Lewis antigens produced in saliva and other secretions are glycoproteins, but Lewis cell-bound antigens absorbed from plasma onto the RBC membranes are glycolipids. The

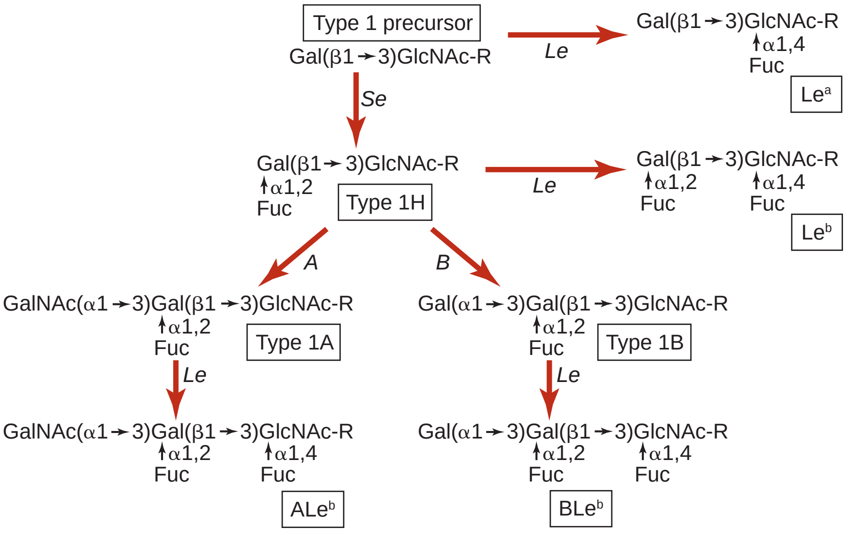

Figure 8–2. Formation of Lewis antigens. Gal = galactose; GlcNAc = *N*-acetylglucosamine; Fuc = fucose; GalNAc = *N*-acetylgalactosamine.

Table 8–8 **Antigens and Phenotypes Resulting From Interaction of Lewis, Secretor, and ABO Genes**

LEWIS, SECRETOR, AND ABO GENES

Genes	Antigens in Secretions	RBC Phenotype
Le, Se, A/B/H	Le^a, Le^b, A, B, H	A, B, H, Le(a – b+)
lele, Se, A/B/H	A, B, H	A, B, H, Le(a – b –)
Le, sese, A/B/H	Le^a	A, B, H, Le(a+b –)
lele, sese, A/B/H	None	A, B, H, Le(a – b –)
Le, sese, hh, A/B	Le^a	O$_h$, Le(a+b –)
Le, Se, hh, A/B	Le^a, Le^b, A, B, H	A*, B*, Le(a – b+)

*para-Bombay

gastrointestinal tract is thought to be the primary source of Lewis glycolipid in plasma.[5] Le(a–b–) RBCs incubated with plasma from Le(a+) or Le(b+) individuals can be converted to Le(a+b–) or Le(a–b+), respectively. With saliva as a source of Lewis substances, Le(a–b–) RBCs cannot be converted into Lewis-positive phenotypes because Lewis substances in saliva, being glycoproteins, are not adsorbed onto the RBC membranes.

Development of Lewis Antigens

Depending on the genes inherited, Le^a and Le^b glycoproteins will be present in the saliva of newborns, but Lewis glycolipids are not detectable in the plasma until about 10 days after birth. As a result, cord blood and RBCs from newborn infants phenotype as Le(a–b–). Some can be shown to be weakly Le(a+) when tested with a potent anti-Le^a or with methods more sensitive than direct agglutination. Lewis antigens will start to appear shortly after birth, with Le^a developing first when the *Le* gene is present. The Lewis fucosyltransferase is more active than the secretor fucosyltransferase in newborns, so more type 1 chains are available for conversion to Le^a. As the secretor transferase activity increases, converting type 1 to type 1H, Le^b will be detected. In children who inherit both *Le* and *Se* genes, the transformation can be followed from the Le(a–b–) phenotype at birth to Le(a+b–) after 10 days to Le(a+b+) and finally to Le(a–b+), the true Lewis phenotype, after about 6 years. In contrast, children who inherit *Le* and *sese* genes phenotype as Le(a–b–) at birth and transform to Le(a+b–) after 10 days; the Le(a+b–) phenotype persists throughout life. Individuals with *lele* genes phenotype as Le(a–b–) at birth and for the rest of their lives.

Other Lewis Antigens

Le^{ab} is present on all Le(a+b–) and Le(a–b+) RBCs and on 90% of cord RBCs. The antigen was previously known as Le^x, but in 1998 the ISBT renamed it Le^{ab}.[9] Anti-Le^{ab} is fairly common and is frequently found with anti-Le^a or anti-Le^b. The antibody is heterogenous and occurs mainly in Le(a–b–) secretors of group A$_1$, B, or A$_1$B. The antigens now known as Le^x and Le^y are products of *FUT3* on type 2 precursor chains and are not associated with the RBC surface and are not part of the Lewis blood group.[9]

ALe^b and BLe^b result from the addition of the A or B immunodominant sugar, respectively, to type 1H chain in individuals who have at least one *Se* and one *Le* allele. *Se* converts type 1 chains to type 1H, providing a suitable acceptor for the A and B carbohydrates (see **Fig. 8–2**).

The P Blood Group: P1PK (003) and Globoside (028) Systems and Related (209) Antigens

Traditionally, the P blood group comprised the P, P1, and P^k antigens and, later, Luke (LKE). The biochemistry and molecular genetics, although not completely understood as yet, make it clear that at least two biosynthetic pathways and genes at different loci are involved in the development and expression of these antigens. Consequently, these antigens cannot be considered a single blood group system. Currently, in ISBT nomenclature, P1 and P^k are assigned to the P1PK blood group system (003, symbol P1PK), P is assigned to the Globoside blood group system (028, symbol GLOB), and LKE and PX2 are assigned to the Globoside collection (209, symbol GLOB). The reader will notice the confusing use of GLOB as the symbol for both a system and a collection. For simplicity in this chapter, these antigens will be referred to as the P blood group.

The P blood group was introduced in 1927 by Landsteiner and Levine. In their search for new antigens, they injected rabbits with human RBCs and produced an antibody, initially called *anti-P*, that divided human RBCs into two groups: P+ and P–.[7]

In 1951, Levine and colleagues[10] described anti-Tj^a (now known as anti-PP1P^k), an antibody to a high-prevalence antigen that Sanger[11] later showed was related to the P blood group. Because anti-Tj^a defined an antigen common to P+ and P– cells and was made by an apparent P null individual, the original antigen and phenotypes were renamed. Anti-P became anti-P1; the P+ phenotype became P$_1$; the P– phenotype became P$_2$; and the rare P null individual became p.

The P blood group became more complex in 1959 when Matson and coworkers[12] described a new antigen, P^k. This antigen is expressed on all RBCs except those of the very rare p phenotype, but it is not readily detected unless P is absent (i.e., in the P$_1^k$ and P$_2^k$ phenotypes).

The phenotypes, antigens, and antibodies associated with the P blood group are summarized in Table 8–9. There are two common phenotypes: P$_1$ and P$_2$, and three rare phenotypes: p, P$_1^k$, and P$_2^k$. The P$_1$ phenotype describes RBCs that react with anti-P1 and anti-P; the P$_2$ phenotype describes RBCs that do not react with anti-P1 but do react with anti-P. When RBCs are tested only with anti-P1 and not with anti-P, the phenotype should be written as P1+ (or P$_1$) or P1–. Only when P1– RBCs are tested and found to be reactive with anti-P should they be designated as phenotype P$_2$. RBCs of the p phenotype do not react with anti-P1, anti-P, or anti-P^k. RBCs of the P$_1^k$ phenotype

Table 8–9 P Blood Group: Phenotypes, Antigens, and Antibodies

PHENOTYPE	ANTIGENS PRESENT	POSSIBLE ANTIBODIES	PREVALENCE	
			Whites	Blacks
P_1	P1, P, P^k*	None	79%	94%
P_2	P, P^k*	Anti-P1	21%	6%
p	None	Anti-PP1P^k	Rare	Rare
P_1^k	P1, P^k	Anti-P	Very rare	Very rare
P_2^k	P^k	Anti-P, anti-P1	Very rare	Very rare

*Trace amounts of P^k antigen, not detectable by agglutination test

react with anti-P1 and anti-P^k but not with anti-P. RBCs of the P_2^k phenotype react with anti-P^k but not with anti-P1 or anti-P.

Individuals with the p phenotype (P null) are very rare: 5.8 in a million. P nulls are slightly more common in Japan, North Sweden, and in an Amish group in Ohio.[1]

The antibodies generally fall into two categories: clinically insignificant or potently hemolytic.

Basic Concepts

The P blood group antigens, like the ABH antigens, are synthesized by sequential action of glycosyltransferases, which add sugars to precursor substances. The precursor of P1 can also be glycosylated to type 2H chains, which carry ABH antigens. P1, P, or P^k may be found on RBCs, lymphocytes, granulocytes, and monocytes; P can be found on platelets, epithelial cells, and fibroblasts. P and P^k have also been found in plasma as glycosphingolipids and as glycoproteins in hydatid cyst fluid.[7] The antigens have not been identified in secretions. RBCs carry approximately 14 × 10^6 copies of globoside, the P structure, per adult RBC and about 5 × 10^5 copies of P1.[9]

The P blood group antigens are resistant to treatment with ficin and papain, DTT, chloroquine, and glycine-acid EDTA. Reactivity of the antibodies can be greatly enhanced by testing with enzyme-treated RBCs.

The P1 Antigen

The P1 antigen is poorly expressed at birth and may take up to 7 years to be fully expressed.[7] Antigen strength in adults varies from one individual to another: RBCs from some P1+ individuals are P1 strong (P1+^{s}) and others are P1 weak (P1+^{w}). These differences may be controlled genetically or may represent homozygous versus heterozygous inheritance of the gene coding for P1. The strength of P1 can also vary with race. Blacks have a stronger expression of P1 than whites. The rare dominant gene for the In(lu) type Lu(a–b–) RBCs, discussed in the Lutheran section, inhibits the expression of P1 so that P_1 individuals who inherit this modifier gene may type serologically as P1–.

The P1 antigen deteriorates rapidly on storage. When older RBCs are typed or used as controls for typing reagents or when older RBCs are used to detect anti-P1 in serum, false-negative reactions may result.

Anti-P1

Anti-P1 is a common, naturally occurring IgM antibody in the sera of P1– individuals. Anti-P1 is typically a weak, cold-reactive saline agglutinin optimally reactive at 4°C and not seen in routine testing. Stronger examples react at room temperature, and rare examples react at 37°C and bind complement, which is detected in the antiglobulin test when polyspecific (anti-IgG plus anti-C3) reagents are used. Antibody activity can be neutralized or inhibited with soluble P1 substance. If room temperature incubation is not included, antibody activity can often be bypassed altogether. Examples of anti-P1 that react only at temperatures below 37°C can be considered clinically insignificant.

Because P1 antigen expression on RBCs varies and deteriorates during storage, antibodies may react only with RBCs that have the strongest expression and give inconclusive patterns of reactivity when antibody identification is performed. When anti-P1 is suspected, incubating tests at room temperature or lower or pretreating test cells with enzymes can enhance reactions to confirm specificity. Providing units that are crossmatch-compatible at 37°C and the antiglobulin phase, without typing for P1, is an acceptable approach to transfusion.

Rare examples of anti-P1 that react at 37°C can cause in vivo RBC destruction; both immediate and delayed HTRs have been reported.[13] Anti-P1 is usually IgM; IgG forms are rare. HDFN is not associated with anti-P1, presumably because the antibody is usually IgM and the antigen is so poorly developed on fetal RBCs.

Biochemistry

Advanced Concepts

The RBC antigens of the P blood group exist as glycosphingolipids. As with ABH, the antigens result from the sugars added sequentially to precursor structures. Biochemical analyses have shown that the precursor substance for P1 is also a precursor for type 2H chains that carry ABH antigens. However, the genes responsible for the formation of the P1 and ABH antigens are independent.

There are two distinct pathways for the synthesis of the P blood group antigens, as shown in Figure 8–3. The common precursor is lactosylceramide (or Gb2, also known as ceramide dihexose or CDH). The pathway on the figure's left results in the formation of paragloboside and P1. Paragloboside is also the type 2 precursor for ABH. The pathway shown on the figure's right side leads to the production of the globoside series: P^{k}, P, and Luke (LKE).

Genetics

The gene encoding the enzyme responsible for the synthesis of P^{k}, a 4-α-galactosyltransferase (Gb3 or P^{k} synthase), was cloned independently by three research groups in 2000.[14] The gene (*B3GALNT1*) encoding the 3-β-*N*-acetylgalactosaminyltransferase (Gb4 synthase) that is responsible for converting P^{k} to P was also cloned in 2000.[14] Several mutations in both genes have been identified that result in the p and P^{k} phenotypes. A polymorphism in the P^{k} synthase was recently identified that ties the P1 and P^{k} antigens together at the genetic level; consequently, the P system (003), to which the P1 antigen was assigned, was renamed P1PK.[3]

The *P1PK* gene (located at chromosome 22q11.2) and the *P* gene (located at chromosome 3q26.1) are inherited independently. The gene for the synthesis of LKE has not yet been cloned.

Other Sources of P1 Antigen and Antibody

The discovery of strong anti-P1 in two P1− individuals infected with *Echinococcus granulosus* tapeworms led to the identification of P1 and P^{k} substance in hydatid cyst fluid. This fluid was subsequently used in many of the studies that identified the biochemical structures of the P blood group. Strong antibodies to P1 have also been found in patients with fascioliasis (bovine liver fluke disease) and in bird handlers.

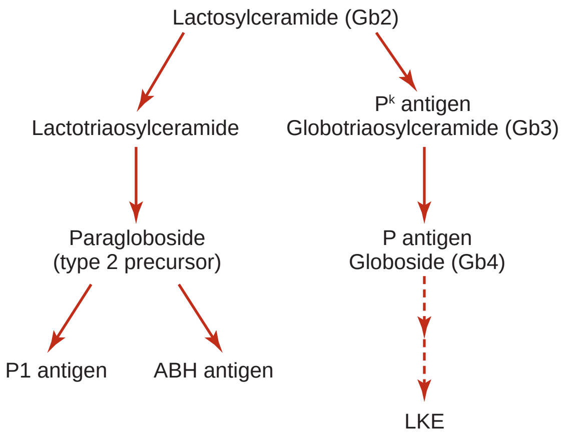

Figure 8–3. Biosynthetic pathways of the P blood group antigens.

Soluble P1 substances have potential use in the blood bank and are commercially available. When it is necessary to confirm antibody specificity or to identify underlying antibodies, these substances can be used to neutralize anti-P1.

Anti-PP1P^{k}

Originally called anti-Tj^{a}, anti-PP1P^{k} was first described in the serum of Mrs. Jay, a p individual with adenocarcinoma of the stomach.[10] Her tumor cells carried P system antigens, and the antibody was credited as having cytotoxic properties that may have helped prevent metastatic growth postsurgery (the T in the Tj^{a} refers to *tumor*).

Anti-PP1P^{k} is produced by p individuals early in life without RBC sensitization and reacts with all RBCs except those of the p phenotype. Unlike antibodies made by other blood group null phenotypes, the anti-P, anti-P1, and anti-P^{k} components of anti-PP1P^{k} are separable through adsorption.[7] Components of anti-PP1P^{k} have been shown to be IgM and IgG.[7] They react over a wide thermal range and efficiently bind complement, which makes them potent hemolysins. Anti-PP1P^{k} has the potential to cause severe HTRs and HDFN.

The antibody is also associated with an increased incidence of spontaneous abortions in early pregnancy. Although the reason for this is not fully known, it has been suggested that having an IgG anti-P component is an important factor. Women with anti-P and anti-PP1P^{k} and a history of multiple abortions have successfully delivered infants after multiple plasmaphereses to reduce their antibody level during pregnancy.[15]

Alloanti-P

In addition to being a component of the anti-PP1P^{k} in p individuals (see above), anti-P is found as a naturally occurring alloantibody in the sera of P^{k} individuals. Its reactivity is similar to that of anti-PP1P^{k} in that it is usually a potent hemolysin reacting with all cells except the autocontrol and those with the p phenotype. However, it differs from anti-PP1P^{k} in that it does not react with cells that have the extremely rare P^{k} phenotype, and the individual making the antibody may type P1+. Alloanti-P is rarely seen, but because it is hemolytic with a wide thermal range of reactivity, it is very significant in transfusion. IgG class anti-P may occur and has been associated with habitual early abortion.

Autoanti-P Associated With Paroxysmal Cold Hemoglobinuria

Anti-P specificity is also associated with the cold-reactive IgG autoantibody in patients with paroxysmal cold hemoglobinuria (PCH). Historically, this rare autoimmune disorder was seen in patients with tertiary syphilis; it now more commonly presents as a transient, acute condition secondary to viral infection, especially in young children. The IgG autoantibody in PCH is described as a biphasic hemolysin: In vitro, the antibody binds to RBCs in the cold, and, via complement activation, the coated RBCs lyse as they are warmed to 37°C. The autoantibody typically does not react in routine test systems but is demonstrable only by the Donath-Landsteiner test. The etiology and diagnosis of PCH are more fully discussed in Chapter 20, "Autoimmune Hemolytic Anemias."

Antibodies to Compound Antigens

Considering the biochemical relationship of the P blood group antigens to ABH and I, it is not surprising that antibodies requiring more than one antigenic determinant have been described, including anti-IP1, -iP1, -I^{T}P1, and -IP. Most examples are cold-reactive agglutinins.

Luke (LKE) Antigen

In 1965, Tippett and colleagues[16] described an antibody in the serum of a patient with Hodgkin's lymphoma that divided the population into three phenotypes: 84% tested Luke+, 14% were weakly positive or Luke(w), and 2% were Luke–. Although this Mendelian-dominant character segregated independently of the P blood group, it was thought to be phenotypically related because the antibody reacted with all RBCs except 2% of P_1 and P_2 phenotypes and those having the rare p and P^k phenotypes. All individuals with the p and P^k phenotype are Luke–.

Disease Associations

Several pathological conditions associated with the P blood group antigens have been described: parasitic infections are associated with anti-P1, early abortions with anti-PP1P^k or anti-P, and PCH with autoanti-P. The P system antigens also serve as receptors for P-fimbriated uropathogenic *E. coli*—a cause of urinary tract infections. The P^k antigen is a receptor for shiga toxins, which cause shigella dysentery and *E. coli*–associated hemolytic uremic syndrome. In addition, P is the receptor of human parvovirus B19. Recent studies demonstrate that P^k provides some protection against HIV infection of peripheral blood mononuclear cells.[17]

The I (027) System and I Antigen

The existence of cold agglutinins in the serum of normal individuals and in patients with acquired hemolytic anemia has long been recognized. In 1956, Wiener and coworkers[2,7] gave a name to one such agglutinin, calling its antigen *I* for "individuality." The antibody reacted with most blood specimens tested. The few nonreactive I– specimens were thought to be from homozygotes for a rare gene producing the "i" antigen; the I– phenotype in adults is now called *adult i*. In 1960, Marsh and Jenkins[18] reported finding anti-i, and the unique relationship between I and i began to unfold.

I and i are not antithetical antigens. Rather, they are branched and linear carbohydrate structures, respectively, that are formed by the action of glycosyl transferases. The gene encoding the transferase that converts i active straight chains into I active branched chains has been cloned, and several mutations responsible for the rare adult i phenotype have been identified.[19] The synthesis of i antigen is not controlled by this same gene. Consequently, I has been raised to blood group system status (system number 027, symbol I) and the i antigen remains in the Ii collection (collection number 207, symbol I). The reader will notice the confusing use of the same ISBT symbol, capital I, for both the I system and Ii collection; in ISBT terminology, the antigen numbers provide the distinction between the I (027001) and i (207002) antigens.

The I and i Antigens

Basic Concepts

The antigens are best introduced by classic serologic facts. Both I and i are high-prevalence antigens, but they are expressed in a reciprocal relationship that is developmentally regulated. At birth, infant RBCs are rich in i; I is almost undetectable. During the first 18 months of life, the quantity of i slowly decreases as I increases until adult proportions are reached; adult RBCs are rich in I and have only trace amounts of i antigen.

There is no true I– or i– phenotype. The strength of I and i varies from individual to individual, and the relative amount detected will depend on the example of anti-I or anti-i used. Data suggest that i reactivity on RBCs is inversely proportional to marrow transit time and RBC age in circulation.

Some people appear not to change their i status after birth. They become the rare adult i. Adult i RBCs generally express more i antigen than do cord RBCs. A spectrum of Ii phenotypes and their characteristic reactivity are shown in Table 8–10.

Treatment of RBCs with ficin and papain enhances reactivity of the I and i antigens with their respective antibodies. The I and i antigens are resistant to treatment with DTT and glycine-acid EDTA.

Anti-I

Anti-I is a common autoantibody that can be found in virtually all sera, although testing at 4°C and/or against enzyme-treated RBCs may be required to detect the reactivity.[1] Consistently strong agglutination with adult RBCs and weak or no agglutination with cord or adult i RBCs define its classic activity (see **Table 8–10**).

Autoanti-I, found in the serum of many normal healthy individuals, is benign—that is, not associated with in vivo

Table 8–10 I and i Antigens

PHENOTYPE	STRENGTH OF REACTIVITY WITH		
	Anti-I	Anti-i	Anti-I^T
Adult I	Strong	Weak	Weak
Cord	Weak	Strong	Strong
Adult i	Weak	Strong	Weakest

RBC destruction. It is usually a weak, naturally occurring, saline-reactive IgM agglutinin with a titer less than 64 at 4°C. Stronger examples agglutinate test cells at room temperature and bind complement, which can be detected in the antiglobulin test if polyspecific reagents are used. Some examples may react only with the strongest I+ RBCs and give inconsistent reactions with panel RBCs.

Incubating tests in the cold enhances anti-I reactivity and helps confirm its identity; albumin and enzyme methods also enhance anti-I reactivity. Testing enzyme-treated RBCs with slightly acidified serum may even promote hemolysis. Occasionally, benign cold autoanti-I can cause problems in pretransfusion testing. Usually, avoiding room temperature testing and using anti-IgG instead of a polyspecific antihuman globulin help to eliminate detection of cold reactive antibodies that may bind complement at lower temperatures. Cold autoadsorption to remove the autoantibody from the serum may be necessary for stronger examples; cold autoadsorbed plasma or serum can also be used in ABO typing.

Pathogenic autoanti-I (e.g., the type associated with cold agglutinin syndrome) typically consists of strong IgM agglutinins with higher titers and a broad thermal range of activity, reacting up to 30° or 32°C. When peripheral circulation cools in response to low ambient temperatures, these antibodies attach in vivo and cause autoagglutination and peripheral vascular occlusion (acrocyanosis) or hemolytic anemia. Refer to Chapter 20 for more information.

Pathogenic anti-I typically reacts with adult and cord RBCs equally well at room temperature and at 4°C, and antibody specificity may not be apparent unless the serum is diluted or warmed to 30°C or 37°C. Potent cold autoantibodies can also mask clinically significant underlying alloantibodies and can complicate pretransfusion testing. Procedures to deal with these problems are discussed in Chapters 9 and 20.

The production of autoanti-I may be stimulated by microorganisms carrying I-like antigen on their surface. Patients with *M. pneumoniae* often develop strong cold agglutinins with I specificity and can experience a transient episode of acute abrupt hemolysis just as the infection begins to resolve. Alloanti-I exists as an IgM or IgG antibody in the serum of most individuals with the adult i phenotype. Although adult i RBCs are not totally devoid of I, the anti-I in these cases does not react with autologous RBCs. It has been traditional to transfuse compatible adult i units to these people, although such practice may be unnecessary, especially when the antibody is not reactive at 37°C.[1] Technologists must be aware that strong autoanti-I can mimic alloanti-I: if enough autoantibody and complement are bound to a patient's RBCs, blocking the antigenic sites, they may falsely type I-negative.

Anti-I is not associated with HDFN because the antibody is IgM, and the I antigen is poorly expressed on infant RBCs.

Anti-i

Alloanti-i has never been described. Autoanti-i is a fairly rare antibody that gives strong reactions with cord RBCs and adult i RBCs and weaker reactions with adult I RBCs. Most examples of autoanti-i are IgM and react best with saline-suspended cells at 4°C. Only very strong examples of autoanti-i are detected in routine testing because standard test cells (except cord RBCs) have poor i expression (see **Table 8–10**).

Unlike anti-I, autoanti-i is not seen as a common antibody in healthy individuals. Potent examples are associated with infectious mononucleosis (Epstein-Barr virus infections) and some lymphoproliferative disorders. High-titer autoantibodies with a wide thermal range may contribute to hemolysis, but because i expression is generally weak they seldom cause significant hemolysis. IgG anti-i has also been described and has been associated with HDFN.[1]

Biochemistry and Genetics

Advanced Concepts

An early association of I and i to ABH was demonstrated by complex antibodies involving both ABH and Ii specificity (see the "Antibodies to Compound Antigens" section). I and i antigens are precursors for the synthesis of ABO and Lewis antigens, and thus they are internal structures on these oligosaccharide chains.

ABH and Ii determinants on the RBC membrane are carried on type 2 chains that attach either to proteins or to lipids. See Figure 8–4 for examples of glycolipid structures for i and I antigens. The i antigen activity is defined by at least two repeating *N*-acetyllactosamine [Gal(β1-4)GlcNAc(β1-3)] units in linear form. I antigen activity is associated with a branched form of i antigen. The *IGnT* (also known as *GCNT2*) gene on chromosome 6p24 encodes the *N*-acetylglucosaminyltransferase, which adds GlcNAc to form the branches.[19,20]

In summary, fetal, cord, and adult i RBCs carry predominantly unbranched chains and have the i phenotype. Normal adult cells have more branched structures and express I antigen. The gene responsible for I antigen (*IGnT*) codes for the branching enzyme. Family studies show that the adult i phenotype is recessive. Heterozygotes (e.g., children inheriting I from one parent and i from the other parent) have intermediate I antigen expression. Several gene mutations have been identified that result in the adult i phenotype.

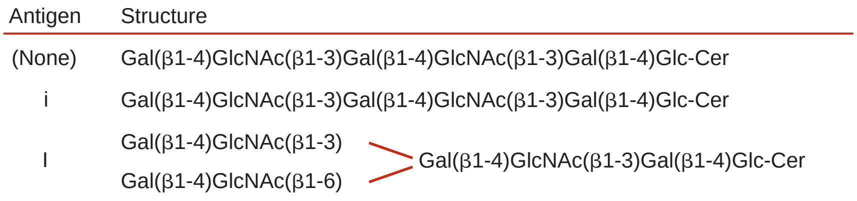
Antigen
Structure
(None)
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc-Cer
i
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc-Cer
I
Gal(β1-4)GlcNAc(β1-3)
Gal(β1-4)GlcNAc(β1-6)
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc-Cer

Figure 8–4. The linear and branched structures carrying i and I activity.

Other Sources of I and i Antigen

I and i antigens are found on the membranes of leukocytes and platelets in addition to RBCs. It is quite likely that the antigens exist on other tissue cells, much like ABH, but this has not been confirmed.

I and i have also been found in the plasma and serum of adults and newborns and in saliva, human milk, amniotic fluid, urine, and ovarian cyst fluid. The antigens in secretions do not correlate with RBC expression and are thought to develop under separate genetic control. For example, the quantity of I antigen in the saliva of adult i individuals and newborns is quite high.

The I^T Antigen and Antibody

In 1965, Curtain and coworkers[21] reported a cold agglutinin in Melanesians that did not demonstrate classical I or i specificity. In 1966, Booth and colleagues[22] confirmed these observations and carefully described the agglutinin's reactivity. This agglutinin reacted strongly with cord RBCs, weakly with normal adult RBCs, and most weakly with adult i RBCs. They concluded that the agglutinin recognizes a transition state of i into I and designated the specificity I^T (*T* for "transition"). However, detection of I^T on fetal RBCs ranging in age from 11 to 16 weeks does not support this hypothesis.[23] This benign IgM anti-I^T was frequently found in two populations: Melanesians and the Yanomama Indians in Venezuela. Whether it is associated with an organism or parasite in these regions is unknown. Examples of IgM and IgG anti-I^T reacting preferentially at 37°C have also been found in patients with warm autoimmune hemolytic anemia, with a special association with Hodgkin's disease.[23]

Antibodies to Compound Antigens

Many other I-related antibodies have been described: anti-IA, -IB, -IAB, -IH, -iH, -IP1, -I^TP1, -IHLe^b, and -iHLe^b. Bearing in mind the close relationship of I to the biochemical structures of ABH, Lewis, and P antigens, it is not surprising to find antibodies that recognize compound antigens. These specificities are not mixtures of separable antibodies; rather, both antigens must be present on the RBCs for the antibody to react. For example, anti-IA reacts with RBCs that carry both I and A but will not react with group O, I+, or group A adult i RBCs. (Table 8–11 summarizes some common cold autoantibodies.) Anti-IH is commonly encountered in the serum of group A_1 individuals. Anti-IH reacts stronger with group O and group A_2 RBCs than with group A_1 RBCs. Anti-IH should be suspected when serum from a group A individual directly agglutinates all group O RBCs but is compatible with most group A donor units.

Disease Associations

Well-known associations between strong autoantibodies and disease or microorganisms have already been discussed: anti-I with cold agglutinin syndrome and *M. pneumoniae*, and anti-i with infectious mononucleosis.

Diseases can also alter the expression of I and i antigens on RBCs. Conditions associated with increased i antigen on RBCs include those with shortened marrow maturation time or dyserythropoiesis: acute leukemia, hypoplastic anemia, megaloblastic anemia, sideroblastic anemia, thalassemia, sickle cell disease, paroxysmal nocturnal hemoglobinuria (PNH), and chronic hemolytic anemia.[1,7] Except in some cases of leukemia, the increase in i on RBCs is not usually associated with a decrease in I antigen; the expression of I antigen can appear normal or sometimes enhanced. Chronic dyserythropoietic anemia type II or hereditary erythroblastic multinuclearity with a positive acidified serum test (HEMPAS) is associated with much greater i activity on RBCs than control cord RBCs. HEMPAS RBCs are very susceptible to lysis with both anti-i and anti-I, and lysis by anti-I appears to be the result of increased antibody uptake and increased sensitivity to complement.[1] In Asians, the adult i phenotype has been associated with congenital cataracts.[20]

The MNS (002) System

Following the discovery of the ABO blood group system, Landsteiner and Levine began immunizing rabbits with human RBCs, hoping to find new antigen specificities. Among the antibodies recovered from these rabbit sera were anti-M and anti-N, both of which were reported in 1927.[7] Data from family studies suggested that M and N were

Table 8–11 **Typical Reactions of Some Cold Autoantibodies***

ANTIBODY	A_1 ADULT	A_2 ADULT	B ADULT	O ADULT	O CORD	A CORD	O_h ADULT	O i ADULT
Anti-I	++++	++++	++++	++++	0/+	0/+	++++	(0)
Anti-i	0/+	0/+	0/+	0/+	++++	++++	0/+	++++
Anti-H	0/+	++	+++	++++	+++	0/+	(0)	+++
Anti-IH	0/+	++	+++	++++	0/+	0/+	(0)	(0)
Anti-IA	++++	+++	0/+	0/+	0/+	0/+	(0)	(0)

*Reactions vary with antibody strength; very potent examples may need to be diluted before specificity can be determined.
0 = negative; + = positive

antithetical antigens. In 1947, after the implementation of the antiglobulin test, Walsh and Montgomery discovered S, a distinct antigen that appeared to be genetically linked to M and N. Its antithetical partner, s, was discovered in 1951. Family studies (and later, molecular genetics) demonstrated the close linkage between the genes controlling M, N, and S, s antigens. There is a disequilibrium in the expression of S and s with M and N. In whites, the common haplotypes were calculated to appear in the following order of relative frequency: *Ns* > *Ms* > *MS* > *NS*.[1,7] The prevalence of the common MN and Ss phenotypes are listed in Table 8–12.

In 1953, an antibody to a high-prevalence antigen, U (for almost *universal* distribution), was named by Weiner. The observation by Greenwalt and colleagues[24] that all U– RBCs were also S–s– resulted in the inclusion of U into the system.

Forty-six antigens have been included in the MNS system, making it almost equal to Rh in size and complexity (Table 8–13). Most of these antigens are of low prevalence and were discovered in cases of HDFN or incompatible crossmatch. Others are high-prevalence antigens. Antibodies to these low- and high-prevalence antigens are not commonly encountered in the blood bank. The genes encoding the MNS antigens are located on chromosome 4.

The MNS blood group system has been assigned the ISBT number 002 (symbol MNS), second after ABO.

M and N Antigens

Basic Concepts

The M and N antigens are found on a well-characterized glycoprotein called **glycophorin A** (GPA), the major RBC **sialic acid–**rich glycoprotein (sialoglycoprotein, SGP). The M and N antigens are antithetical and differ in their amino acid residues at positions 1 and 5 (Fig. 8–5). M is defined by serine at position 1 and glycine at position 5; N has leucine and glutamic acid at these positions, respectively. The antibody reactivity may also be dependent on adjacent carbohydrate chains, which are rich in sialic acid. There are about 10^6 copies of GPA per RBC.[13] The antigens are well developed at birth.

Because M and N are located at the outer end of GPA, they are easily destroyed by the routine blood bank enzymes ficin, papain, and bromelin and by the less common enzymes trypsin and pronase. The antigens are also destroyed by ZZAP, a combination of DTT and papain or ficin, but they are not affected by DTT alone, 2-aminoethylisothiouronium bromide (AET), α-chymotrypsin, chloroquine, or glycine-acid EDTA treatment. Treating RBCs with neuraminidase, which cleaves sialic acid (also known as *neuraminic acid* or *NeuNAc*), abolishes reactivity with only some examples of antibody. M and N antibodies are heterogeneous; some may recognize only specific amino acids, but others recognize both amino acids and carbohydrate chains.

Table 8–12 Prevalence of Common MN and Ss Phenotypes

PHENOTYPE	WHITES (%)	BLACKS (%)
M+N–	28	26
M+N+	50	44
M–N+	22	30
S+s–	11	3
S+s+	44	28
S–s+	45	69
S–s–U–	0	< 1

S and s Antigens

S and s antigens are located on a smaller glycoprotein called **glycophorin B** (GPB) that is very similar to GPA (see "Biochemistry" section and **Fig. 8–5**). S and s are differentiated by the amino acid at position 29 on GPB. Methionine defines S, whereas threonine defines s. The epitope may also include the amino acid residues at position 34 and 35 and the carbohydrate chain attached to threonine at position 25.[7] There are fewer copies of GPB (about 200,000) than GPA per RBC.[7] In addition, there are about 1.5 times more copies of GPB on S+s– RBCs than on S–s+ RBCs.[1] S and s also are well developed at birth.

S and s antigens are less easily degraded by enzymes because the antigens are located farther down the glycoprotein, and enzyme-sensitive sites are less accessible. Ficin, papain, bromelin, pronase, and chymotrypsin can destroy S and s activity, but the amount of degradation may depend on the strength of the enzyme solution, the length of treatment, and the enzyme-to-cell ratio. Trypsin does not destroy the S and s antigens, and neither does DTT, AET, chloroquine, or glycine-acid EDTA treatment.

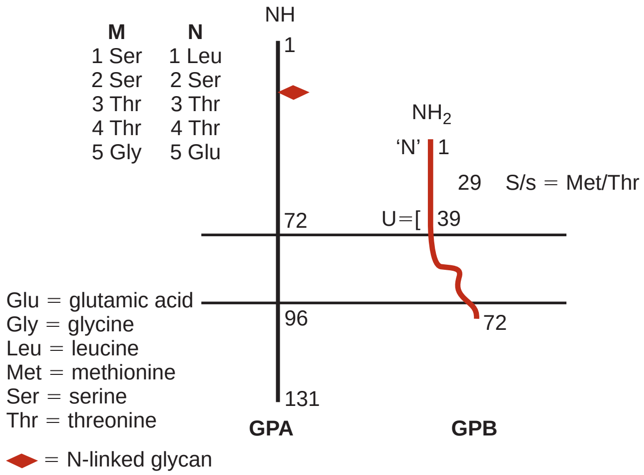

Figure 8–5. Comparison of glycophorin A (GPA) and glycophorin B (GPB).

Table 8–13 **Summary of MNS Antigens**

ANTIGEN NAME	ISBT NUMBER	PREVALENCE (%)	YEAR DISCOVERED
M	MNS1	Polymorphic	1927
N	MNS2	Polymorphic	1927
S	MNS3	Polymorphic	1947
s	MNS4	Polymorphic	1951
U	MNS5	High	1953
He	MNS6	Low	1951
Mi^{a}	MNS7	Low	1951
M^{c}	MNS8	Low	1953
Vw	MNS9	Low	1954
Mur	MNS10	Low	1961
M^{g}	MNS11	Low	1958
Vr	MNS12	Low	1958
M^{e}	MNS13	Low	1961
Mt^{a}	MNS14	Low	1962
St^{a}	MNS15	Low	1962
Ri^{a}	MNS16	Low	1962
Cl^{a}	MNS17	Low	1963
Ny^{a}	MNS18	Low	1964
Hut	MNS19	Low	1966
Hil	MNS20	Low	1966
M^{v}	MNS21	Low	1966
Far	MNS22	Low	1968
s^{D}	MNS23	Low	1978
Mit	MNS24	Low	1980
Dantu	MNS25	Low	1982
Hop	MNS26	Low	1977
Nob	MNS27	Low	1977
En^{a}	MNS28	High	1969
ENKT	MNS29	High	1986
'N'	MNS30	High	1977
Or	MNS31	Low	1964
DANE	MNS32	Low	1991
TSEN	MNS33	Low	1992
MINY	MNS34	Low	1992
MUT	MNS35	Low	1992
SAT	MNS36	Low	1991

Continued

Table 8–13 Summary of MNS Antigens—cont'd

ANTIGEN NAME	ISBT NUMBER	PREVALENCE (%)	YEAR DISCOVERED
ERIK	MNS37	Low	1993
Os^a	MNS38	Low	1983
ENEP	MNS39	High	1995
ENEH	MNS40	High	1993
HAG	MNS41	Low	1995
ENAV	MNS42	High	1996
MARS	MNS43	Low	1992
ENDA	MNS44	High	2005
ENEV	MNS45	High	2006
MNTD	MNS46	Low	2006

Anti-M

Many examples of anti-M are naturally occurring saline agglutinins that react below 37°C. Although we may think of agglutinating anti-M as IgM, 50% to 80% are IgG or have an IgG component.[1] They do not bind complement, regardless of their immunoglobulin class, and they do not react with enzyme-treated RBCs. Anti-M appears to be more common in children than in adults and is particularly common in patients with bacterial infections.[7]

Because of antigen **dosage**, many examples of anti-M may react better with M+N– RBCs (genotype *MM*) than with M+N+ RBCs (genotype *MN*). Very weak anti-M may not react with M+N+ RBCs at all, making antibody identification difficult. Antibody reactivity can be enhanced by increasing the serum-to-cell ratio or incubation time, or both, by decreasing incubation temperature or by adding a potentiating medium such as albumin, low ionic strength saline solution (LISS), or polyethylene glycol (PEG).

Some examples of anti-M are pH-dependent, reacting best at pH 6.5. These antibodies may be detected in plasma (which is slightly acidic from the anticoagulant) but not in unacidified serum.[1] Other examples of anti-M react only with RBCs exposed to glucose solutions. Such antibodies react with M+ reagent RBCs or donor RBCs stored in preservative solutions containing glucose but do not react with freshly collected M+ RBCs.

As long as anti-M does not react at 37°C, it is not clinically significant for transfusion and can be ignored. When anti-M reacts at 37°C, it is sufficient to provide units that are crossmatch-compatible at 37°C and at the antiglobulin phase without typing for M antigen. Anti-M rarely causes HTRs, decreased red cell survival, or HDFN.

Anti-N

The serologic characteristics of the common anti-N (made by individuals whose RBCs type M+N– and S+ or s+) are similar to those of anti-M: a cold-reactive IgM or IgG saline agglutinin that does not bind complement or react with enzyme-treated RBCs. Anti-N can demonstrate dosage, reacting better with M–N+ (*NN*) RBCs than with M+N+ (*MN*) RBCs. Rare examples are pH- or glucose-dependent.

Also like anti-M, anti-N is not clinically significant unless it reacts at 37°C. It has been implicated only with rare cases of mild HDFN.[1] Anti-N is less common than anti-M. A potent anti-N can be made by the rare individual whose RBCs type M+N–S–s– because they lack both N and GPB that has 'N' activity (see the "Biochemistry" section).

Anti-N was also seen in renal patients, regardless of their MN type, who were dialyzed on equipment sterilized with formaldehyde. Dialysis-associated anti-N reacts with any N+ or N– RBCs treated with formaldehyde and is called anti-N^f. Formaldehyde may alter the M and N antigens so that they are recognized as foreign. The antibody titer decreases when dialysis treatment and exposure to formaldehyde stops. Because anti-N^f does not react at 37°C, it is clinically insignificant for transfusion.

Anti-S and Anti-s

Most examples of anti-S and anti-s are IgG, reactive at 37°C and the antiglobulin test. A few express optimal reactivity between 10°C and 22°C by saline indirect antiglobulin test. If anti-S or anti-s specificity is suspected but the pattern of reactivity is not clear, incubating tests at room temperature and immediately performing the antiglobulin test (without incubating at 37°C) may help in identification. Dosage effect can be exhibited by many examples of anti-S and anti-s, although it may not be as dramatic as seen with anti-M and anti-N.[1]

The antibodies may or may not react with enzyme-treated RBCs, depending on the extent of treatment and the efficiency of the enzyme. Although seen less often than anti-M, anti-S and anti-s are more likely to be clinically significant. They may bind complement, and they have been implicated in severe HTRs with hemoglobinuria. They have also caused HDFN.

Units selected for transfusion must be antigen-negative and crossmatch-compatible. Because only 11% of whites and 3% of blacks are s–, it can be difficult to provide blood for a patient with anti-s. S– units are much easier to find (45% of whites and 69% of blacks are S–). Antibodies to low-prevalence antigens are commonly found in reagent anti-S; these can cause discrepant antigen typing results.

Biochemistry

Advanced Concepts

GPA, the structure carrying the M and N antigens, is a single-pass membrane SGP that consists of 131 amino acids. The hydrophilic NH_2 terminal end, which lies outside the RBC membrane, has 72 amino acid residues, 15 *O*-glycosidically linked oligosaccharide chains (GalNAc-serine/threonine), and 1 *N*-glycosidic chain (sugar-asparagine). The portion that traverses the membrane is hydrophobic and contains 23 amino acids. The hydrophilic COOH end, which contains 36 amino acids and no carbohydrates, lies inside the membrane and interacts weakly with the membrane cytoskeleton.

GPB, the structure carrying the S, s, and U antigens, consists of 72 amino acids and 11 *O*-linked oligosaccharide chains and no *N*-glycans. It has an outer glycosylated portion of 44 amino acids, a hydrophobic portion of 20 amino acids that traverses the RBC membrane, and a short cytoplasmic "tail" of 8 amino acids.

The first 26 amino acids on GPB are identical to the first 26 amino acids on the N form of GPA (GPA^N). This N activity of GPB is denoted as 'N' (N-quotes) to distinguish it from the N activity of GPA^N. Anti-N reagents are formulated to not recognize the weak reactivity of the 'N' structure. The U antigen, expressed when normal GPB is present, is located very close to the RBC membrane (see the "U– Phenotype" section).

Most *O*-linked carbohydrate structures on GPA and GPB are branched tetrasaccharides containing one GalNAc, one Gal, and two NeuNAc (sialic acid). NeuNAc helps give the RBC its negative charge. About 70% of the RBC NeuNAc is carried by GPA, and about 16% is carried by GPB.

GPA associates with protein band 3, which affects the expression of the antigen Wr^b of the Diego blood group system (located on protein band 3). GPB appears to be associated with the Rh protein and Rh-associated glycoprotein complex as evidenced by the greatly reduced S and s expression on Rh_{null} RBCs.[7]

Other antigens within the MNS system have been evaluated biochemically and at the molecular level. Some are associated with altered GPA because of amino acid substitutions or changes in carbohydrate chains. Others are expressed on variants of GPA or GPB. Still others result from a genetic event that encodes a hybrid glycophorin that has parts of both GPA and GPB. The altered glycophorins are associated with changes in glycosylation, changes in molecular weight, loss of high-prevalence antigens or the appearance of novel low-prevalence antigens, or alterations in the expression of MNS antigens.[1,7,9]

RBCs that lack GPA or GPB are not associated with disease or decreased RBC survival (see the "Disease Associations" section). GPA and GPB are expressed on renal endothelium and epithelium.[9]

Genetics

The genes *GYPA* and *GYPB*, which code for GPA and GPB, respectively, are located on chromosome 4 at 4q28–q31. The known alleles for *GYPA* (*M/N*) and *GYPB* (*S/s*) are codominant. The genes are highly homologous (meaning they are very similar) and probably arose by gene duplication. *GYPA* is considered to be the ancestral gene.[25]

GYPA is organized into seven exons. *GYPB* has a size and arrangement similar to those of *GYPA* but has only five coding exons plus one noncoding or pseudoexon. A third highly homologous gene, *GYPE*, does not appear to make a glycoprotein that has been definitively recognized on the RBC surface, but it participates in gene rearrangements that result in variant alleles.

Misalignment of *GYPA* and *GYPB* during meiosis, followed by an unequal crossing over, appears to explain some of the variant glycophorins observed in the MNS system. The resulting new reciprocal *GYP*(*A-B*) and *GYP* (*B-A*) genes encode GP(A-B) and GP(B-A) hybrid glycophorins, respectively (Fig. 8–6).[7,26] The point of fusion between the GPA and GPB part in the hybrid glycophorin can give rise to novel antigens (e.g., antigen of low prevalence).

For more complex variant glycophorins, a gene conversion event probably occurs. Gene conversion involves a nonreciprocal exchange of genetic material from one gene to another homologous gene (Fig. 8–7).[7,26] As with hybrids resulting from crossing over, the new amino acid sequence at the junction of the hybrid glycophorin can give rise to novel antigens of low prevalence. Also, expected antigens may be missing if the coding exons are replaced by the inserted genetic material.

GPA- and GPB-Deficient Phenotypes

RBCs of three rare phenotypes lack GPA or GPB or both GPA and GPB; consequently, they lack all MNS antigens that are normally expressed on those structures.

U– Phenotype

The U antigen is located on GPB very close to the RBC membrane between amino acids 33 and 39 (see **Fig. 8–5**).

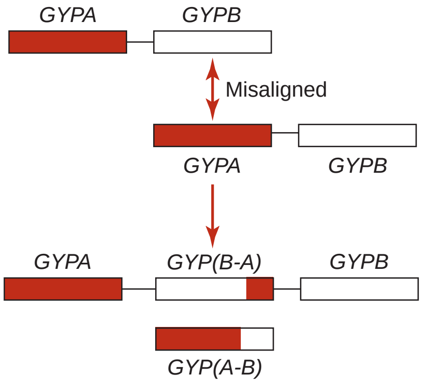

Figure 8–6. Depiction of how misalignment during meiosis can lead to a crossover and reciprocal *GYP(B-A)* and *GYP(A–B)* hybrids.

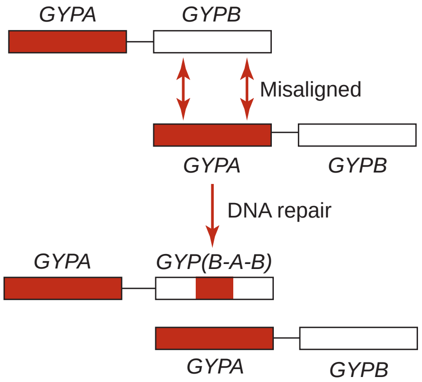

Figure 8–7. In a gene conversion event, nucleotides from one strand of DNA are transferred to the misaligned homologous gene. If this is the coding strand, a hybrid glycophorin will be formed; the partner chromosome will be repaired and carry the naïve (unaltered) *GYPA* and *GYPB* genes.

This high-prevalence antigen is found on RBCs of all individuals except about 1% of African Americans (and 1% to 35% of Africans) who lack GPB because of a partial or complete deletion of *GYPB*. The RBCs usually type S–s–U–, and these individuals can make anti-U in response to transfusion or pregnancy. Anti-U is typically IgG and has been reported to cause severe and fatal HTRs and HDFN.

The U antigen is resistant to enzyme treatment; thus, most examples of anti-U react equally well with untreated and enzyme-treated RBCs. However, there are rare examples of broadly reactive anti-U that do not react with papain-treated RBCs.[1]

Some examples of anti-U react with apparent U– RBCs, although weakly, by adsorption and elution.[7] Such RBCs are said to be U variant (U^{var}); these have an altered GPB that does not express S or s. There is a strong correlation between the low-prevalence antigen He, found in about 3% of African Americans, and U^{var} expression.[27]

Because examples of anti-U are heterogeneous, U– units selected for transfusion must be crossmatched to determine compatibility. Some patients may tolerate U^{var} units; others may not. Many examples of anti-U are actually anti-U plus anti-GPB. If the patient is U– and N–, the antibody may actually be a potent anti-N plus anti-U, making the search for compatible blood more difficult.

En(a–) Phenotype

In 1969, Darnborough and coworkers[28] and Furuhjelm and colleagues[29] described an antibody to the same high-prevalence antigen, called En^a (for *envelope*), which reacted with all RBCs except those of the propositi. In both of these cases, the En(a–) individuals appeared to be M–N– with reduced NeuNAc on their RBCs. The RBCs of the two individuals were mutually compatible.

Most En(a–) individuals produce anti-En^a, which is an umbrella term for reactivity against various portions of GPA unrelated to M or N, but not all antibodies detect the same portion. Anti-En^aTS recognizes a trypsin-sensitive (TS) area on GPA between amino acids 20 and 39, anti-En^aFS reacts with a ficin-sensitive (FS) area between amino acids 46 and 56, and anti-En^aFR reacts with a ficin-resistant (FR) area around amino acids 62 to 72.[1,7]

Although the gene responsible for this phenotype has been termed *En*, it is now known that the En(a–) phenotype has more than one origin. Most often, the En(a–) phenotype results from homozygosity for a rare gene deletion at the *GYPA* locus; consequently, no GPA is produced, but GPB is not affected. This type of En(a–) inheritance is called En(a–)Fin, representing the type described in the Finnish report.[29] However, the En(a–) phenotype in the English report[28] probably represents heterozygosity for a hybrid gene along with the very rare M^k gene; this type is often called En(a–)UK. Several other En(a–) phenotypes have been reported that result from homozygosity for a variant allele or inheritance of two dissimilar variant alleles.

Anti-En^a can be confused with two other specificities that react with all normal RBCs—anti-Pr and anti-Wr^b. Anti-Pr does not react with enzyme-treated RBCs and can be confused with anti-En^aFS; anti-Wr^b does not react with En(a–) RBCs but does react with enzyme-treated RBCs and can be confused with anti-En^aFR. Anti-En^a has caused severe HTRs and HDFN. It is extremely difficult and may be impossible to find units compatible for patients with anti-En^a; siblings are a potential source of compatible blood if they are also ABO and Rh compatible.

M^k Phenotype

The rare silent gene M^k was named by Metaxas and Metaxas-Buhler in 1964 when they found an allele that did not produce M or N.[7] A second family showed that M^k was also silent at the *Ss* locus. Several other M^k heterozygotes have been found. In 1979, two related M^kM^k blood donors were found in Japan. The RBCs of these individuals typed M–N–S–s–U–En(a–)Wr(a–b–), but they had a normal hematologic picture. More individuals have since been identified, and it is now known that the M^k gene represents a single, near-complete deletion of both *GYPA* and *GYPB*[7]; thus, M^kM^k is the null phenotype in the MNS system. The M^kM^k genotype is associated with decreased RBC sialic acid content but increased glycosylation of RBC membrane bands 3 and 4.1.

Other Antibodies in the MNS System

Antibodies to antigens other than M, N, S, and s are rarely encountered and can usually be grouped into two categories: those directed against low-prevalence antigens and those directed against high-prevalence antigens.

Antibodies to high-prevalence antigens are easily detected with antibody detection RBCs. Antibodies to low-prevalence antigens are rarely detected by the antibody detection test but are seen as an unexpected incompatible crossmatch or an unexplained case of HDFN. Few hospital blood banks have the test cells available to identify the specificity, but enzyme reactivity and MNS antigen typing may offer clues.

When antibodies to low-prevalence antigens are encountered, it is common practice to transfuse units that are crossmatch-compatible at 37°C and in the antiglobulin phase. Typing sera for MNS antigens other than M and N and S and s are not generally available, so the antigen status of compatible RBCs can seldom be confirmed. Also, when one antibody to a low-prevalence antigen is found, antibodies to other low-prevalence antigens are also frequently present in the same serum. If the antibody is directed to a high-prevalence antigen, the assistance of an immunohematology reference laboratory is generally needed to identify the antibody and obtain appropriate antigen-negative units.

Autoantibodies

Autoantibodies to M and N have been reported.[1] Not all examples of anti-M in M+ individuals or anti-N in N+ individuals are autoantibodies. Many fail to react with the patient's own RBCs. It may be that these individuals have altered GPA and that their antibody is specific for a portion of the common antigen they lack. Autoantibodies to U and En^a are more common and may be associated with warm-type autoimmune hemolytic anemia.

Disease Associations

GPA^M may serve as the receptor by which certain pyelonephritogenic strains of *E. coli* gain entry to the urinary tract. The malaria parasite *Plasmodium falciparum* appears to use alternative receptors, including GPA and GPB for cell invasion; some of these receptors also involve NeuNAc.

The Kell (006) and Kx (019) Systems

The Kell blood group system consists of 32 high-prevalence and low-prevalence antigens; it was the first blood group system discovered after the introduction of antiglobulin testing. Anti-K was identified in 1946 in the serum of Mrs. Kelleher. The antibody reacted with the RBCs of her newborn infant, her older daughter, her husband, and about 7% of the random population.[7] In 1949, anti-k, the high-prevalence antithetical partner to K, was described. Kell remained a two-antigen system until the antithetical antigens Kp^a and Kp^b were described in 1957 and 1958, respectively. Likewise, Js^a (described in 1958) and Js^b (described in 1963) were found to be antithetical and related to the Kell system. The discovery of the null phenotype in 1957, designated K$_o$, helped associate many other antigens with the Kell system. Antibodies that reacted with all RBCs except those with the K$_o$ phenotype recognized high-prevalence antigens that were phenotypically related.

The 32 antigens included in the Kell blood group system, designated by the symbol KEL or 006 by the ISBT, are listed in Table 8–14. In 1961, a numerical notation was proposed for the Kell system. As new antigens were discovered, some received only a number for the antigen name. However, the traditional notation persisted and is more useful in conveying antithetical relationships (e.g., K and Js^a). Consequently, the commonly used nomenclature for Kell antigens is a mixture of symbols using letters and numbers. The associated antigen Kx is the only antigen in the Kx system, ISBT number 019 and symbol XK.

Table 8–14 **Kell System Antigens**

NAME	NUMERIC TERMINOLOGY	ISBT NUMBER	PREVALENCE (%)	ANTITHETICAL ANTIGEN(S)	YEAR DISCOVERED
K	1	KEL1	9	K	1946
k	2	KEL2	99.8	K	1949
Kp^a	3	KEL3	2 (whites)	Kp^b, Kp^c	1957
Kp^b	4	KEL4	> 99.9	Kp^a, Kp^c	1958
Ku	5	KEL5	> 99.9		1957
Js^a	6	KEL6	< 0.1 whites 20 blacks	Js^b	1958
Js^b	7	KEL7	> 99.9 whites 99 blacks	Js^a	1963
Ul^a	10	KEL10	< 3 Finns		1968
Côté	11	KEL11	> 99.9	K17	1971
Boc	12	KEL12	> 99.9		1973
SGRO	13	KEL13	> 99.9		1973
San	14	KEL14	> 99.9	K24	1973
k-like	16	KEL16	99.8		1976
Wk^a	17	KEL17	0.3	K11	1974
VM	18	KEL18	> 99.9		1975

Continued

Table 8–14 Kell System Antigens—cont'd

NAME	NUMERIC TERMINOLOGY	ISBT NUMBER	PREVALENCE (%)	ANTITHETICAL ANTIGEN(S)	YEAR DISCOVERED
Sub	19	KEL19	> 99.9		1979
Km	20	KEL20	> 99.9		1979
Kp^c	21	KEL21	< 0.1	Kp^a, Kp^b	1945
Ikar	22	KEl22	> 99.9		1982
Centauro	23	KEL23	< 0.01		1987
CL	24	KEL24	< 2	K14	1985
VLAN		KEL25	< 0.1		1996
TOU		KEL26	> 99.9		1995
RAZ	K27	KEL27	> 99.9		1994
VONG		KEL28	< 0.1		2003
KALT		KEL29	> 99.9		2006
KTIM		KEL30	> 99.9		2006
KYO		KEL31	< 0.1		2006
KUCI		KEL32	> 99.9		2007
KANT		KEL33	> 99.9		2007
KASH		KEL34	> 99.9		2007
KELP		KEL35	> 99.9		2010

Obsolete: K8, K9, K15

Basic Concepts

Kell blood group antigens are found only on RBCs. They have not been found on platelets or on lymphocytes, granulocytes, or monocytes. The associated Xk protein is found in erythroid tissues and in other tissues, such as brain, lymphoid organs, heart, and skeletal muscle.[30]

The K antigen can be detected on fetal RBCs as early as 10 weeks and is well developed at birth. The k antigen has been detected at 7 weeks. The total number of K antigen sites per RBC is quite low: only 3,500 up to 18,000 sites per RBC.[9] Despite its lower quantity, K is very immunogenic.

The antigens are not denatured by the routine blood bank enzymes ficin and papain but are destroyed by trypsin and chymotrypsin when used in combination.[7] Thiol-reducing agents, such as 100 to 200 mM DTT, 2-mercaptoethanol (2-ME), AET, and ZZAP (which contains DTT in addition to enzyme), destroy Kell antigens but not Kx. Glycine-acid EDTA (an IgG-removal agent) also destroys Kell antigens.

The prevalence of common Kell phenotypes are listed in Table 8–15. There are five sets of antithetical antigens; antithetical relationships have not been established for the other high- and low-prevalence antigens. Some of the Kell antigens (e.g., Js^b) are more prevalent in certain populations.

Table 8–15 Prevalence of Common Kell System Phenotypes

PHENOTYPE	WHITES (%)	BLACKS (%)
K–k+	91	96.5
K+k+	8.8	3.5
K+k–	0.2	< 0.1
Kp(a+b–)	< 0.1	0
Kp(a+b+)	2.3	Rare
Kp(a–b+)	97.7	100
Js(a+b–)	0	1
Js(a+b+)	Rare	19
Js(a–b+)	100	80

K and k Antigens

Excluding ABO, K is rated second only to D in immunogenicity. Most anti-K appear to be induced by pregnancy and transfusion.[7] Fortunately, the prevalence of K antigen is low (9% in whites), and the chance of receiving a K+ unit is small. If anti-K develops, compatible units are easy to find.

Antibodies to k antigen are seldom encountered. Only 2 in 1,000 individuals lack k and are capable of developing the antibody. The likelihood that these few individuals will receive transfusions and become immunized is even less.

Kp^a, Kp^b, and Kp^c Antigens

Alleles *Kp^a* and *Kp^c* are low-prevalence mutations of their high-prevalence partner *Kp^b*. The Kp^a antigen is found in about 2% of whites. The *Kp^a* gene is associated with suppression of other Kell antigens on the same molecule, including k and Js^b.[7] The effect appears to result from a reduced amount of the Kell glycoprotein (produced by the *Kp^a* allele) inserted in the RBC membrane. The Kp^c antigen is even more rare.

Js^a and Js^b Antigens

The Js^a antigen, antithetical to the high-prevalence antigen Js^b, is found in about 20% of blacks but in fewer than 0.1% of whites.[9] The prevalence of Js^a in blacks is almost 10 times greater than the prevalence of the K antigen in blacks. Js^a and Js^b were linked to the Kell system when it was discovered that K$_o$ RBCs were Js(a–b–).

Anti-K

Outside the ABO and Rh antibodies, anti-K is the most common antibody seen in the blood bank. Anti-K is usually IgG and reactive in the antiglobulin phase, but some examples agglutinate saline-suspended RBCs. The antibody is usually made in response to antigen exposure through pregnancy and transfusion and can persist for many years.

Naturally occurring IgM examples of anti-K are rare and have been associated with bacterial infections. Marsh and colleagues[31] studied an IgM anti-K in an untransfused 20-day-old infant with an *E. coli* O125:B15 infection whose mother did not make anti-K. The organism was shown to have a somatic K-like antigen that reacted with the infant's antibody, so the bacterial antigen was thought to have been the stimulus. The antibody disappeared after recovery.

Some examples of anti-K react poorly in methods incorporating low-ionic media, such as LISS, and in some automated systems. The most reliable method of detection is the indirect antiglobulin test. The potentiating medium, PEG, may increase reactivity.

Anti-K has been implicated in severe HTRs. Although some examples of anti-K bind complement, in vivo RBC destruction is usually extravascular via the macrophages in the spleen. Anti-K is also associated with severe HDFN. The antibody titer does not always accurately predict the severity of disease; stillbirth has been seen with anti-K titers as low as 64. Fetal anemia in anti-K HDFN is associated with suppression of erythropoiesis due to destruction of erythroid precursor cells, which can be additional to destruction of circulating antigen-positive RBCs, as seen in anti-D HDFN. Kell glycoprotein is expressed on fetal RBCs at a much earlier stage of erythropoiesis than Rh antigens.[32] When a pregnant woman is identified as making anti-K, it is prudent to type the father for the K antigen. If he's K+, the fetus should be monitored carefully for signs of HDFN.

Antibodies to Kp^a, Js^a, and Other Low-Prevalence Kell Antigens

Antibodies to the low-prevalence Kell antigens are rare because so few people are exposed to these antigens. Because routine antibody detection RBCs do not carry low-prevalence antigens, the antibodies are most often detected through unexpected incompatible crossmatches or cases of HDFN.

The serologic characteristics and clinical significance of these antibodies parallel anti-K. The original anti-Kp^a was naturally occurring, but most antibodies result from transfusion or pregnancy.

Antibodies to k, Kp^b, Js^b, and Other High-Prevalence Kell Antigens

Antibodies to high-prevalence Kell system antigens are rare because so few people lack these antigens. They also parallel anti-K in serologic characteristics and clinical significance.

The high-prevalence antibodies are easy to detect but difficult to work with, because most blood banks do not have the antigen-negative panel cells needed to exclude other alloantibodies, nor do they have typing reagents to phenotype the patient's RBCs. Testing an unidentified high-prevalence antibody against DTT- or AET-treated RBCs is a helpful technique: Reactivity that is abolished with DTT or AET treatment suggests that the antibody may be related to the Kell system and enables the technologist to exclude common alloantibodies. Caution is needed before assigning Kell system specificity until antigen-negative RBCs are tested, because DTT also denatures JMH and high-prevalence antigens in the LW, Lutheran, Dombrock, Cromer, and Knops systems. Finding compatible units for transfusion can be difficult; siblings and rare-donor inventories are the most likely sources. Patients with antibodies to high-prevalence antigens should be encouraged to donate autologous units and, if possible, to participate in a rare-donor program.

Biochemistry

Advanced Concepts

The Kell antigens are located on a glycoprotein that consists of 731 amino acids and spans the RBC membrane once. The N-terminal domain is intracellular, and the large external C-terminal domain is highly folded by disulfide linkages (Fig. 8–8). The Kell glycoprotein is covalently linked with another protein, called Xk, by a single disulfide bond. The Xk protein (440 amino acids) is predicted to span the RBC membrane ten times. Kell antigen expression is dependent upon the presence of the Xk protein.

The Kell glycoprotein is a member of the neprilysin (M13) family of zinc endopeptidases associated with the cleavage of big endothelins, but how this relates to the physiological role of the Kell glycoprotein remains unclear. The structure of the Xk protein suggests a membrane transport protein. The absence of Xk results in McLeod syndrome (see the "McLeod Phenotype and Syndrome" section below).

Genetics

The *KEL* gene, located on chromosome 7 (at 7q34), is organized into 19 exons of coding sequence. Single base mutations encoding amino acid substitutions are responsible for the different Kell antigens. Several different mutations (e.g., point, frameshift, or splice site mutations) have been found that result in the rare null phenotype K_o.[7,9]

No Kell haplotype has been shown to code for more than one low-prevalence antigen. People who test positive for two low-prevalence Kell antigens have always been found to carry the encoding alleles on opposite chromosomes. For example, someone who types Kp(a+) and Js(a+) is genetically kKp^aJs^b on one chromosome and kKp^bJs^a on the other.

The *XK* gene, which encodes the Kx antigen, is independent of *KEL* and is located on the short arm of the X chromosome at position Xp21.1.[7]

The Kx Antigen

Kx is present on all RBCs except those of the rare McLeod phenotype (see the "McLeod Phenotype and Syndrome" section below). K_o and K_{mod} phenotype RBCs have increased Kx antigen.[7] When Kell antigens are denatured with AET or DTT, the expression of Kx increases.

The K_o Phenotype and Anti-Ku(K5)

K_o RBCs lack expression of all Kell antigens. K_o RBCs have no membrane abnormality and survive normally in circulation. The phenotype is rare; data suggest a frequency of 1:25,000 in whites.[7]

Immunized individuals with the K_o phenotype typically make an antibody called anti-Ku (K5) that recognizes the "universal" Kell antigen (Ku) present on all RBCs except K_o. Anti-Ku appears to be a single specificity and cannot be separated into components. Anti-Ku has caused both HDFN and HTRs.[7]

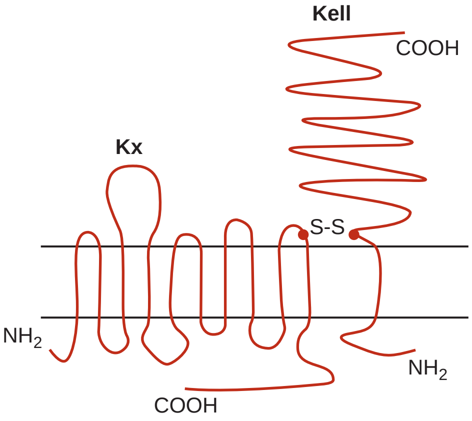

Figure 8–8. Proposed structures for Kell and Kx proteins. The two proteins are linked through one disulfide bond. The conformation of the large external domain of the Kell glycoprotein is unknown; 15 cysteine residues suggest the presence of disulfide bonds and extensive folding.

Because K_o RBCs are negative for k, Kp^b, Js^b, and so forth, they are very useful in investigating complex antibody problems. They can help confirm a Kell system specificity or rule out other underlying specificities. When K_o RBCs are not available, they can be made artificially by treating normal RBCs with DTT, AET, or glycine-acid EDTA.

The McLeod Phenotype and Syndrome

In 1961, Allen and coworkers[33] described a young male medical student who initially appeared to be Kell null but who demonstrated weak expression of k, Kp^b, and Js^b detectable by adsorption-elution methods. This unusual phenotype was called McLeod, after the student.

The McLeod phenotype is very rare. All who have it are male, and inheritance is X-linked through a carrier mother. McLeod phenotype RBCs lack Kx and another high-prevalence antigen, Km, and have marked depression of all other Kell antigens. The weakened expression of the Kell antigens is designated by a superscript *w* for "weak"—for example, K–k+^{w} Kp(a–b+^{w}). The McLeod phenotype has been associated with several mutations and deletions at the *XK* locus.

A significant proportion of the RBCs in individuals with the McLeod phenotype are acanthocytic (having irregular shapes and protrusions) with decreased deformability and reduced in vivo survival. As a result, individuals with the McLeod phenotype have a chronic but often well-compensated hemolytic anemia characterized by reticulocytosis, bilirubinemia, splenomegaly, and reduced serum haptoglobin levels.

Individuals with the McLeod phenotype have a variety of muscle and nerve disorders that, together with the serologic and hematologic picture, are collectively known as the McLeod syndrome, one of the neuroacanthocytosis syndromes. McLeod individuals develop a slow, progressive form of muscular dystrophy between ages 40 and 50 years and cardiomegaly (leading to cardiomyopathy). The associated neurological disorder presents initially as areflexia (a lack of deep tendon reflexes) and progresses to choreiform movements (well-coordinated but involuntary movements). These individuals also have elevated serum creatinine phosphokinase levels of the MM type (cardiac/skeletal muscle) and carbonic anhydrase III levels.

In 1971, Giblett and colleagues made an association between the rare Kell phenotypes, including the McLeod phenotype, and the rare X-linked chronic granulomatous disease (CGD).[34] CGD is characterized by the inability of phagocytes to make NADH oxidase, an enzyme important in generating H_2O_2, which is used to kill ingested bacteria. Afflicted children can die at an early age from overwhelming infections if not treated. Not all males with the McLeod phenotype have CGD, nor do all patients with CGD have the McLeod phenotype.

At one time it was suggested that CGD was caused by a lack of Kx on white blood cells, and several alleles at the *XK* locus were proposed to explain Kx expression on McLeod RBCs and

CGD white blood cells. More recent data have shown that this theory is not valid. The *XK* gene resides on the X chromosome near deletions associated with CGD, Duchenne muscular dystrophy, and retinitis pigmentosa, in the Xp21 region.

The expression of Kx in women who are carriers of the McLeod phenotype follows the Lyon hypothesis, which states that in early embryo development, one X chromosome randomly shuts down in female cells that have two. All cells descending from the resulting cell line express only the allele on the active chromosome. Hence, McLeod carriers exhibit two RBC populations: one having Kx and normal Kell antigens, the other having the McLeod phenotype and acanthocytosis. The percentage of McLeod phenotype RBCs in carriers varies from 5% to 85%.[1]

McLeod males with CGD make anti-Kx + Km, which reacts strongly with K_o RBCs, weaker with normal Kell phenotype RBCs, and not at all with McLeod phenotype RBCs. Anti-Km is made by McLeod males without CGD. There are rare reports of a McLeod male without CGD who has made anti-Kx + Km.[35] The expression of Kell antigens on RBCs with common, McLeod, and K_o phenotypes is summarized in Table 8–16.

Altered Expressions of Kell Antigens

Weaker-than-normal Kell antigen expression is associated with the McLeod phenotype and the suppression by the *Kp^a^* gene (cis-modified effect) on Kell antigens. Depressed Kell antigens are also seen on RBCs with the rare Gerbich-negative phenotypes Ge: –2, –3, 4 and Ge: –2, –3, –4. The phenotypic relationship between Gerbich and Kell is not understood. The umbrella term K_{mod} is used to describe other phenotypes with very weak Kell expression, often requiring adsorption-elution tests for detection. As a group, these RBCs have a reduced amount of Kell glycoprotein and enhanced Kx expression. Some K_{mod} individuals make an antibody that resembles anti-Ku but does not react with other K_{mod} RBCs (unlike anti-Ku made by K_o individuals).

Patients with autoimmune hemolytic anemia, in which the autoantibody is directed against a Kell antigen, may have depressed expression of that antigen. Antigen strength returns to normal when the anemia resolves and the DAT becomes negative. This phenomenon appears to be more common in the Kell system than in others.[1]

Finally, RBCs may appear to acquire Kell antigens. McGinnis and coworkers[36] described a K– patient who acquired a K-like antigen during a *Streptococcus faecium* infection. Cultures containing the disrupted organism converted K– cells to K+ but bacteria-free filtrates did not.

Autoantibodies

Most Kell autoantibodies are directed against undefined high-prevalence Kell antigens, but identifiable autoantibodies to K, Kp^b, and K13 have been reported. Mimicking specificities have also been reported, such as when an apparent anti-K is eluted from DAT+ K– RBCs and the anti-K in the eluate can be adsorbed onto K– RBCs.

The Duffy (008) System

The Duffy blood group system was named for Mr. Duffy, a multiply transfused hemophiliac who in 1950 was found to have the first described example of anti-Fy^a. One year later, the antibody defining its antithetical antigen, Fy^b, was found in the serum of a woman who had had three pregnancies.

In 1955, Sanger and colleagues[37] reported that the majority of African Americans tested were Fy(a–b–). The gene responsible for this null phenotype was called *Fy*. *FyFy* appeared to be a common genotype in blacks, especially in Africa; the gene is exceedingly rare in whites.

In 1975, it was observed that Fy(a–b–) RBCs resist infection in vitro by the monkey malaria organism *Plasmodium knowlesi*. It was later shown that Fy(a–b–) RBCs also resist infection by *P. vivax* (one of the organisms causing malaria in humans).[37] This discovery provides an explanation for the predominance of the Fy(a–b–) phenotype in persons originating from West Africa.

Antibodies to other antigens in the Duffy blood group system, Fy3, Fy5, are rarely encountered. RBCs that are Fy(a–b–) are also Fy: –3, –5. Fy5 is also not present on Rh_{null} RBCs, regardless of the Fy^a or Fy^b status of those RBCs. The Duffy blood group system is designated by the symbol FY or 008 by the ISBT.

Fy^a and Fy^b Antigens

Basic Concepts

The Duffy antigens most important in routine blood bank serology are Fy^a and Fy^b. They can be identified on fetal RBCs as early as 6 weeks gestational age and are well developed at birth. There are about 13,000 to 14,000 Fy^a

Table 8–16 Expression of Kell Antigens on RBCs With Common, K_o, and McLeod Phenotypes

PHENOTYPE	RBC ANTIGEN EXPRESSION			POSSIBLE ANTIBODY
	Kell Antigens	Km	Kx	
Common	k, Kp^b, Js^b, K11 . . .	Normal	Weak	Alloantibody
K_o	None	None	Increased	Anti-Ku
McLeod	Trace k, Kp^a, Js^b, K11 . . .	None	None	Anti-Kx + Km (CGD) Anti-Km (non-CGD)

or Fy^b sites on Fy(a+b–) and Fy(a–b+) RBCs, respectively; there are half that number of Fy^a sites on Fy(a+b+) RBCs.[7] The antigens have not been found on platelets, lymphocytes, monocytes, or granulocytes, but they have been identified in other body tissues, including brain, colon, endothelium, lung, spleen, thyroid, thymus, and kidney cells.[9] The prevalence of the common phenotypes in the Duffy system are given in Table 8–17. The disparity in distribution in different races is notable.

Fy^a and Fy^b antigens are destroyed by common proteolytic enzymes, such as ficin, papain, bromelin, and chymotrypsin, and by ZZAP (which contains either papain or ficin in addition to DTT); they are not affected by DTT alone, AET, or glycine-acid EDTA treatment. Neuraminidase may reduce the molecular weight of Fy^a and Fy^b, but it does not destroy antigenic activity and neither does purified trypsin.

Anti-Fy^a and Anti-Fy^b

Anti-Fy^a is a common antibody and is found as a single specificity or in a mixture of antibodies. Anti-Fy^a occurs three times less frequently than anti-K. Anti-Fy^b is 20 times less common than anti-Fy^a and often occurs in combination with other antibodies. The antibodies are usually IgG and react best at the antiglobulin phase. Rare examples of anti-Fy^a and anti-Fy^b bind complement. A few examples are saline agglutinins. Antibody activity is enhanced in a low ionic strength medium. Because anti-Fy^a and anti-Fy^b do not react with enzyme-treated RBCs, this is a helpful technique when multiple antibodies are present.

Some examples of anti-Fy^a and anti-Fy^b show dosage, reacting more strongly with RBCs that have a double dose than RBCs from heterozygotes. It must be remembered that some reagent RBCs that appear to be from homozygotes (and have a double dose of either Fy^a or Fy^b) may actually be from heterozygotes if they are from black donors; a silent allele, *Fy*, is commonly found in blacks. For example, Fy(a+b–) RBCs will have a double dose of Fy^a if they are from a white *Fy^{a}Fy^a* donor but will have a single dose of Fy^a if they are from a black donor who is genetically *Fy^{a}Fy*. Additional phenotypic markers commonly found in black donors can give a clue to the possible presence of the silent *Fy* allele: R$_o$, S–s–, V+VS+, Js(a+), Le(a–b–).

Table 8–17 Prevalence of Common Duffy Phenotypes

PHENOTYPE	WHITES (%)	AFRICAN AMERICANS (%)	CHINESE (%)
Fy(a+b–)	17	9	90.8
Fy(a+b+)	49	1	8.9
Fy(a–b+)	34	22	0.3
Fy(a–b–)	Very rare	68	0

Anti-Fy^a and anti-Fy^b have been associated with acute and delayed HTRs. Once the antibody is identified, Fy(a–) or Fy(b–) blood must be given; finding such units in a random population is not difficult. For example, one in three random units of blood is Fy(a–) and one in five random units of blood is Fy(b–). Anti-Fy^a and anti-Fy^b are associated with HDFN that ranges from mild to severe.

Rare autoantibodies with mimicking Fy^a and Fy^b specificity have been reported—for example, anti-Fy^b that can be adsorbed onto and eluted from Fy(a+b–) RBCs. Issitt and Anstee[1] suggest that these may represent alloantibodies with "sloppy" specificity made early in an immune response.

Biochemistry

Advanced Concepts

Enzymes, membrane solubilization methods, immunoblotting, radiolabeling, and amino acid sequencing have all been used to study the biochemistry of Duffy antigens.[1] Duffy antigens reside on a glycoprotein of 336 amino acids that has a relative mass of 36 kD and two *N*-glycosylation sites (Fig. 8–9).[38] The glycoprotein is predicted to traverse the cell membrane seven times and has two predicted disulfide bridges.

The amino acid at position 42 on the Duffy glycoprotein defines the Fy^a and Fy^b polymorphism: Fy^a has glycine, and Fy^b has aspartic acid. The Fy3 epitope, as defined by monoclonal antibody, is on the third extracellular loop, and Fy6 appears to involve amino acids 19 through 25.[38]

The Duffy glycoprotein is a member of the superfamily of chemokine receptors and is known as the *Duffy antigen receptor for chemokines* (DARC). Thus, in addition to being a receptor for the malaria parasite *P. vivax*, the Duffy glycoprotein binds a variety of proinflammatory cytokines.

Genetics

In 1968, the Duffy gene was linked to a visible, inherited abnormality of chromosome 1, thus becoming the first human gene to be assigned to a specific chromosome. The gene is located near the centromere on the long arm of chromosome 1 at position 1q23.2. The *Fy* locus is syntenic to the *Rh* locus, which is located near the tip of the short arm; that is, they

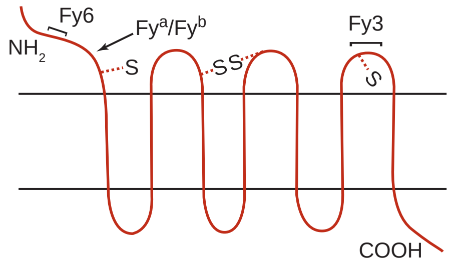

Figure 8–9. Proposed structure for the Duffy protein. Disulfide bonds probably link the NH2 terminal domain and the third loop and the first and second loop.

are on the same chromosome, but they are far enough apart that linkage cannot be demonstrated and serologically they appear to segregate independently.

There are three common alleles at the *Fy* locus: Fy^a, Fy^b, and *Fy*. Fy^a and Fy^b encode the antithetical antigens Fy^a and Fy^b, respectively, and *Fy* is a silent allele and is the major allele in blacks. The *Fy* gene in Fy(a–b–) blacks is an Fy^b variant with a change in the promoter region of the gene, which disrupts the binding site for mRNA transcription in the RBC.[9] Consequently, Fy(a–b–) blacks do not express Fy^b on their RBCs but express Fy^b in other tissues. The presence of Fy^b in tissues presumably precludes the recognition of Fy^b as foreign; thus, no anti-Fy^b is made by these individuals. A molecular analysis of Fy(a–b–) whites revealed different mutations. These individuals carry no Duffy protein on their RBCs or on other tissues and thus can form anti-Fy^b and anti-Fy3.

Typing for Duffy antigens has been performed on the RBCs of chimpanzees, gorillas, and old- and new-world monkeys. The results suggest that *Fy3* developed first, then Fy^b, and that Fy^a arose during human evolution.[9]

Fy^x

Fy^x was described in 1965 as a new allele at the *Fy* locus. It does not produce a distinct antigen but rather is an inherited weak form of Fy^b that reacts with some examples of anti-Fy^b. Fy^x has been described in white populations. Individuals with Fy^x may type Fy(b–), but their RBCs adsorb and elute anti-Fy^b. They also have depressed expression of their Fy3 and Fy5 antigens. The decreased expression of Fy^b due to Fy^x appears to be related to a reduced amount of Duffy glycoprotein on the surface of RBCs.[39] There is no anti-Fy^x.

Fy3 Antigen and Antibody

In 1971, anti-Fy3 was found in the serum of an Fy(a–b–) white Australian female. It reacted with all RBCs tested except those of the Fy(a–b–) phenotype. Because it was an inseparable anti-Fy^{a}Fy^b, it was thought to react with an antigenic determinant or precursor common to both Fy^a and Fy^b and was called Fy3. Unlike Fy^a and Fy^b, the Fy3 antigen is not destroyed by enzymes.

Anti-Fy3 is a rare antibody made by Fy(a–b–) individuals who lack the Duffy glycoprotein. The Fy(a–b–) phenotype has been found in whites, Cree Indian families, and blacks.[1] Blacks with the Fy(a–b–) phenotype rarely make anti-Fy3. Some blacks who make anti-Fy3 initially make anti-Fy^a.[7]

Fy5 Antigen and Antibody

In 1973, Colledge and coworkers[40] discovered anti-Fy5 in the serum of an Fy(a–b–) black child who later died of leukemia. Initially it was thought to be a second example of anti-Fy3, because it reacted with all Fy(a+) or Fy(b+) RBCs but not with Fy(a–b–) cells. The antibody differed in that it reacted with the cells from an Fy(a–b–)Fy:–3 white female, but it did not react with Fy(a+) or Fy(b+) Rh$_{null}$ RBCs and reacted only weakly with Fy(a+) or Fy(b+) D–– RBCs.

Sometimes, sera containing anti-Fy5 also contain anti-Fy^a. Several examples of anti-Fy5 have been reported in multiply transfused Fy(a–b–) sickle cell patients with a mixture of other antibodies.

The molecular structure of Fy5 is not known, but it appears to be the result of interaction between the Rh complex and the Duffy glycoprotein. People who are Fy(a–b–) or Rh$_{null}$ do not make Fy5 antigen and are at risk of making the antibody, although few do. Like Fy3, Fy5 is not destroyed by enzymes.

The Kidd (009) System

The Kidd blood group is a simple and straightforward system consisting of only three antigens. In 1951, Allen and colleagues[41] reported finding an antibody in the serum of Mrs. Kidd, whose infant had HDFN. The antibody, named anti-Jk^a, reacted with 77% of Bostonians. Its antithetical partner, Jk^b, was found 2 years later. The null phenotype Jk(a–b–) was described in 1959. The propositus made an antibody to a high-prevalence antigen called Jk3, which is present on any RBC positive for Jk^a or Jk^b. No other antigens associated with the Kidd system have been described.

The Kidd system is designated by the symbol JK or 009 by the ISBT. It has special significance to routine blood banking because of its antibodies, which can be difficult to detect and are a common cause of HTRs.

Jk^a and Jk^b Antigens

Basic Concepts

Jk^a and Jk^b are antigens commonly found on RBCs of most individuals. Table 8–18 summarizes the prevalence of the four known phenotypes. There are notable differences in antigen frequency among various races: 91% of blacks and 77% of whites are Jk(a+); 57% of blacks and only 28% of whites are Jk(b–).

Jk^a and Jk^b antigens are well developed on the RBCs of neonates. Jk^a has been detected on fetal RBCs as early as 11 weeks; Jk^b has been detected at 7 weeks.[7] Although this early development of Kidd antigens contributes to the potential for HDFN, anti-Jk^a and anti-Jk^b are only rarely responsible for severe HDFN. Jk(a+b–) RBCs carry 14,000 antigen sites per cell.[7] The Kidd antigens are not very immunogenic.

Kidd antigens are not denatured by papain or ficin; treatment of RBCs with enzymes generally enhances reactivity with Kidd antibodies. Kidd antigens are also not affected by chloroquine, DTT, AET, or glycine-acid EDTA. The antigens are not found on platelets, lymphocytes, monocytes, or granulocytes.

Table 8–18 Prevalence of Kidd Phenotypes

PHENOTYPE	WHITES (%)	BLACKS (%)	ASIANS (%)
Jk(a+b–)	28	57	23
Jk(a+b+)	49	34	50
Jk(a–b+)	23	9	27
Jk(a–b–)	Exceedingly rare	Exceedingly rare	0.9 Polynesians

Anti-Jk^a and Anti-Jk^b

Kidd antibodies have a notorious reputation in the blood bank. They demonstrate dosage, are often weak, and are found in combination with other antibodies, all of which make them difficult to detect.

Anti-Jk^a is more frequently encountered than anti-Jk^b, but neither antibody is common. The antibodies are usually IgG (antiglobulin reactive) but may also be partly IgM and are made in response to pregnancy or transfusion.

The ability of Kidd antibodies to show dosage can confound inexperienced serologists. Many anti-Jk^a and anti-Jk^b react more strongly with RBCs that carry a double dose of the respective antigen and may not react with Jk(a+b+) RBCs. An anti-Jk^a that reacts only with Jk(a+b–) RBCs can give inconclusive panel results and appear compatible with Jk(a+b+) cells. Readers are urged to rule out anti-Jk^a and anti-Jk^b only with Jk(a+b–) and Jk(a–b+) panel cells, respectively, and to type all crossmatch-compatible units with commercial antisera. To ensure that antisera can indeed detect weak expressions of the antigen, Jk(a+b+) RBCs should be tested in parallel as the positive control.

Antibody reactivity can also be enhanced by using LISS or PEG (to promote IgG attachment), by using four drops of serum instead of two (to increase the antibody-to-antigen ratio), or by using enzymes such as ficin or papain. In vitro hemolysis can sometimes be observed with enzyme-treated RBCs if serum is tested; antigen dose may influence this hemolytic activity.[1]

Many examples of the Kidd antibodies bind complement. Rare examples are detected only by the complement they bind (i.e., they are nonreactive in antiglobulin tests using anti-IgG reagents). Testing serum (rather than plasma) and using polyspecific reagents with both anti-IgG and anti-complement can be helpful in these situations.[1]

The titer of anti-Jk^a or anti-Jk^b quickly declines in vivo. A strong antibody identified following a transfusion reaction may be undetectable in a few weeks or months.[1] This confirms the need to check blood bank records for previously identified antibodies before a patient is transfused. It is equally important to inform the patient that he or she has such an antibody and to provide a wallet card that notes the specificity in case the patient is transfused elsewhere.

The decline in antibody reactivity and the difficulty in detecting Kidd antibodies are reasons why they are a common cause of HTRs, especially of the delayed type. Although intravascular hemolysis has been noted in severe reactions, coated RBCs more often are removed extravascularly. The rate of clearance of incompatible RBCs can vary but is usually rapid.

Contrary to their hemolytic reputation in transfusion, most Kidd antibodies are only rarely associated with severe cases of HDFN.[42]

Biochemistry

Advanced Concepts

Heaton and McLoughlin[43] reported in 1982 that Jk(a–b–) RBCs resist lysis in 2M urea, a solution commonly used to lyse RBCs in a sample before it is used in some automated platelet-counting instruments. Urea crosses the RBC membrane, causing an osmotic imbalance and an influx of water, which rapidly lyses normal cells. With Jk(a+) or Jk(b+) RBCs, lysis in 2M urea occurs within 1 minute; with Jk(a–b–) cells, lysis is delayed by 30 minutes.[7]

The predicted Kidd glycoprotein has 389 amino acids with 10 membrane-spanning domains and two *N*-glycosylation sites, one of which is extracellular on the third extracellular loop (Fig. 8–10). The glycoprotein is a urea transporter.

Genetics

The *Jk* locus is on chromosome 18 at position 18q12.3. The gene *SLC14A1* (for solute carrier family 14, member 1) is a member of the urea transporter gene family. The gene is organized into 11 exons. The Jk^a/Jk^b polymorphism is associated with an amino acid substitution at position 280, predicted to be located on the fourth extracellular loop of the glycoprotein. Molecular studies have demonstrated the silent *Jk* allele can arise from mutations in both the *Jk^a* and *Jk^b* alleles. *Jk^a* and *Jk^b* are inherited as codominant alleles.

Jk(a–b–) Phenotype and the Recessive Allele, Jk

People with the null Jk(a–b–) phenotype lack Jk^a, Jk^b, and the common antigen Jk3. Although very rare, the Jk(a–b–) phenotype is most abundant among Polynesians, and it has also been identified in Filipinos, Indonesians, Chinese, and Japanese.[1] The null phenotype has also been reported in several European families (Finnish, French, Swiss, and English) and in the Mato Grosso Indians of Brazil. The delayed lysis

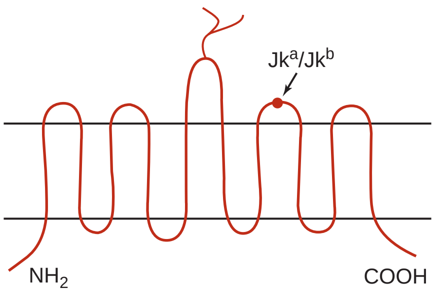

Figure 8–10. Proposed structure for Kidd protein. One of two proposed *N*-glycans is extracellular and is located on the third extracellular loop; the Jk^a/Jk^b polymorphism is located on the fourth extracellular loop.

of Jk(a–b–) RBCs in 2M urea has proved an easy way to screen families and populations for this rare phenotype.

No clinical abnormalities have been associated with the Jk(a–b–) phenotype to date. Several unrelated Jk(a–b–) individuals had normal blood urea nitrogen, creatinine, and serum electrolytes, but studies on two individuals with this phenotype showed a marked defect in their ability to concentrate urine.

Family studies show that most Jk(a–b–) nulls are homozygous for the rare "silent" allele *Jk*. Parents of *JkJk* offspring and children of *JkJk* parents type Jk(a+b–) or Jk(a–b+) but never Jk(a+b+), because they are genetically $Jk^{a}Jk$ or $Jk^{b}Jk$. Their RBCs also demonstrate a single dose of Jk^{a} or Jk^{b} antigen in titration studies.

Jk(a–b–) Phenotype and the Dominant In(Jk) Allele

Another genetic explanation for the Jk(a–b–) phenotype is association with a dominant gene called *In*(*Jk*), for "inhibitor," that shows a dominant pattern of inheritance within a Japanese family analogous to the inhibitor gene responsible for the dominant type Lu(a–b–) phenotype in the Lutheran blood group system.[7,9] Dominant type Jk(a–b–) RBCs adsorb and elute anti-Jk3 and anti-Jk^{a} or anti-Jk^{b} (depending on which genes were inherited), indicating that the antigens are expressed but only very weakly. Individuals with the dominant type Jk(a–b–) phenotype do not make anti-Jk3. Family studies show that the *In*(*Jk*) gene does not reside at the *Jk* locus. The molecular basis is unknown.

Anti-Jk3

Alloanti-Jk3 is an IgG antiglobulin-reactive antibody that looks like an inseparable anti-$Jk^{a}Jk^{b}$. Because panel cells are Jk(a+) or Jk(b+), anti-Jk3 reacts with all RBCs tested except the autocontrol. Most blood banks do not have the rare cells needed to confirm anti-Jk3; however, they can easily determine its most probable specificity by means of antigen typing. The individual making the antibody will type Jk(a–b–). Like other Kidd antibodies, anti-Jk3 reacts optimally by an antiglobulin test, and the reactivity is enhanced with enzyme pretreatment of the RBCs.

Anti-Jk3 has been associated with severe immediate and delayed HTRs and with mild HDFN. Compatible units are best found by typing siblings or searching the rare donor files.

Autoantibodies

Autoantibodies with Kidd specificity (anti-Jk^{a}, anti-Jk^{b}, and anti-Jk3) are rare, but they have been associated with autoimmune hemolytic anemia.[1] As with other blood groups, Kidd autoantibodies may have mimicking specificity or may be associated with depressed antigen expression.

The Lutheran (005) System

In 1945, anti-Lu^{a} was found (and described in detail a year later) in the serum of a patient with lupus erythematosus, following the transfusion of a unit of blood carrying the corresponding low-prevalence antigen.[44] (This patient also made anti-c, anti-N, the first example of anti-C^{W}, and anti-Levay, now known as Kp^{c}!) The new antibody was named Lutheran for the donor; the donor's last name was Lutteran but the donor blood sample was incorrectly labeled.[2] In 1956, Cutbush and Chanarin[45] described anti-Lu^{b}, which defined the antithetical partner to Lu^{a}.

The blood group system appeared complete until 1961, when Crawford and colleagues[46] described the first Lu(a–b–) phenotype. Unlike most null phenotypes at the time, this one demonstrated dominant inheritance. In 1963, the Lu(a–b–) phenotype inherited as a recessive silent allele was described.

Twenty antigens are part of the Lutheran system, numbered through Lu22; some antigens are also known by other common names. Two numbers (Lu10 and Lu15) are obsolete. Most of these antigens are high prevalence; four sets of antigens are antithetical. Many of the antigens were associated with the Lutheran system when their corresponding antibodies were nonreactive with the rare Lu(a–b–) RBCs. All are summarized in Table 8–19. The ISBT designation of the Lutheran blood group system is LU or 005.

Basic Concepts

Blood bankers seldom deal with the serology of the Lutheran blood group system. The antigens are either high prevalence, so only a few people lack the antigen and can make an alloantibody, or very low prevalence, so that only a few people are ever exposed. Consequently, the antibodies are seen infrequently, and there are not much data on the clinical significance of Lutheran antibodies.

Although the antigens have been detected on fetal RBCs as early as 10 to 12 weeks of gestation, they are poorly developed at birth. As a result, HDFN is rare and only mild.[9] Lutheran antigens have not been detected on platelets, lymphocytes, monocytes, or granulocytes. However, Lutheran glycoprotein is widely distributed in tissues: brain, lung, pancreas, placenta, skeletal muscle, and hepatocytes (especially fetal hepatic epithelial cells).[7] The presence of Lutheran glycoprotein on placental tissue may result in adsorption of maternal antibodies to Lutheran antigens, thus decreasing the likelihood of HDFN.[9]

Lutheran antigens are resistant to the enzymes ficin and papain and to glycine-acid EDTA treatment but are destroyed by treatment with the enzymes trypsin and α-chymotrypsin. Most Lutheran antibodies do not react with RBCs treated with the sulfhydryl reagents DTT and AET.

Lu^{a} and Lu^{b} Antigens

Lu^{a} and Lu^{b} are antigens produced by allelic codominant genes. The prevalence of common phenotypes are listed in Table 8–20. Most individuals are Lu(b+); 8% of whites and 5% of blacks are Lu(a+).[9]

Lutheran antigen expression is variable from one individual to another. The number of Lu^{b} sites per RBC is low, estimated to be from 1,640 to 4,070 on Lu(a–b+) RBCs and from 850 to 1,820 on Lu(a+b+) RBCs.[7]

Table 8–19 Lutheran System Antigens

CONVENTIONAL NAME	PREVALENCE (%)	YEAR DISCOVERED	COMMENTS
Lu^a	8 whites 5 blacks	1945	Antithetical to Lu^b
Lu^b	99.8	1956	Antithetical to Lu^a
Lu3	> 99.9	1963	
Lu4	> 99.9	1971	
Lu5	> 99.9	1972	
Lu6	> 99.9	1972	Antithetical to Lu9
Lu7	> 99.9	1972	
Lu8	> 99.9	1972	Antithetical to Lu14
Lu9	2	1973	Antithetical to Lu6
Lu11	> 99.9	1974	
Lu12	> 99.9	1973	
Lu13	> 99.9	1983	
Lu14	2.4	1977	Antithetical to Lu8
Lu16	> 99.9	1980	
Lu17	> 99.9	1979	
Au^a, Lu18	80 whites	1961	Antithetical to Au^b
Au^b, Lu19	50 whites	1989	Antithetical to Au^a
Lu20	> 99.9	1992	
Lu21	> 99.9	2002	
Lu22	> 99.9	2009	LURC

Table 8–20 Prevalence of Common Lutheran Phenotypes

PHENOTYPE	MOST POPULATIONS (%)
Lu (a+b–)	0.15
Lu (a+b+)	7.5
Lu (a–b+)	92.35
Lu (a–b–)	Very rare

Anti-Lu^a

Most examples of anti-Lu^a are IgM naturally occurring saline agglutinins that react better at room temperature than at 37°C. A few react at 37°C by indirect antiglobulin test. Some are capable of binding complement, but in vitro hemolysis has not been reported.

Anti-Lu^a often goes undetected in routine testing because most reagent RBCs are Lu(a–). Anti-Lu^a is more likely encountered as an incompatible crossmatch or during an antibody workup for another specificity. Experienced technologists recognize Lutheran antibodies by their characteristic loose, mixed-field reactivity in a test tube. Examples of anti-Lu^a that react only at temperatures below 37°C are clinically insignificant. There are no documented cases of immediate HTRs; there are only rare and mild delayed HTRs due to anti-Lu^a.[1]

Anti-Lu^b

Although the first example of anti-Lu^b was a room-temperature agglutinin, and IgM and IgA antibodies have been noted, most examples of anti-Lu^b are IgG and reactive at 37°C at the antiglobulin phase. The antibody is made in response to pregnancy or transfusion.

Alloanti-Lu^b reacts with all cells tested except the autocontrol, and reactions are often weaker with Lu(a+b+) RBCs and cord RBCs. Anti-Lu^b has been implicated with shortened survival of transfused cells and post-transfusion jaundice, but severe or acute hemolysis has not been reported.

Biochemistry

Advanced Concepts

The Lutheran antigens are located on a type 1 transmembrane protein. The protein exists in two forms as a result of alternative RNA splicing: the longer Lu glycoprotein and the shorter basal cell adhesion molecule (B-CAM). The longer 85-kD protein contains 597 amino acids with five extracellular domains, a hydrophobic transmembrane domain of 19 amino acids, and a cytoplasmic domain of 59 amino acids (Fig. 8–11). The smaller isoform (78 kD) is identical except for a shorter cytoplasmic domain of 59 amino acids. The external portion consists of five disulfide-bonded domains. The Lutheran glycoproteins belong to the immunoglobulin superfamily of proteins; the repeating extracellular domains are homologous to immunoglobulin variable or constant domains. The Lutheran proteins are multifunctional adhesion molecules that bind laminin, notably in sickle cell disease.[47]

The molecular basis for the four pairs of antithetical antigens and several of the high-prevalence antigens has been determined through the creation of Lutheran glycoprotein mutants and subsequent sequencing of the exons encoding the extracellular domains. For most of the antigens, expression was caused by a single nucleotide polymorphism, resulting in single amino acid changes on the protein level.[48]

Genetics

The *Lu* gene is located on chromosome 19 at position 19q13.2, along with genes that govern expression of several blood group antigens (*H*, *Se*, *Le*, *LW*, *Ok*^{a}) . A linkage between *Lu* and the *Se* gene (*FUT2*) was the first example of autosomal linkage described in humans.

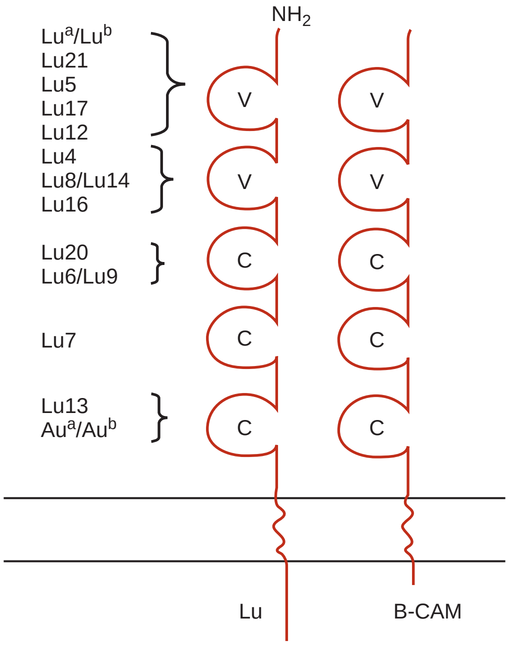

Figure 8–11. The two structures encoded by the Lu gene: the longer Lu glycoprotein and the shorter basal cell adhesion molecule (B-CAM). The five extracellular disulfide-bonded domains are homologous to immunoglobulin variable (V) or constant (C) domains.

Lu(a–b–) Phenotypes

Three genetic explanations for the Lu(a–b–) phenotype have been described. These are summarized in Table 8–21.

Dominant Type Lu(a–b–)

The first Lu(a–b–) family study was reported by the propositus herself.[46] Because the phenotype was seen in successive generations in 50% of her family members and others, and because null individuals passed normal Lutheran genes to their offspring, the expression of Lutheran was thought to be suppressed by a rare dominant regulator gene later called *In*(*Lu*) for “inhibitor of Lutheran.” Recently, mutations in the gene for Erythroid Krüppel-like Factor (EKLF), a transcription factor, were shown to be associated with the In(Lu) phenotype in 21 of 24 In(Lu) individuals studied.[49] In all cases, the mutated *EKLF* allele occurred in the presence of a normal *EKLF* allele. The authors of this study concluded that the In(Lu) phenotypeis caused by inheritance of a loss-of-function mutation on one allele of *EKLF*.

Dominant-type Lu(a–b–) RBCs carry trace amounts of Lutheran antigens as shown by adsorption-elution studies. For example, the RBCs of a person with the dominant type Lu(a–b–) who inherited two normal *Lu*^{b} genes will type Lu(a–b–) with routine methods but will adsorb and elute anti-Lu^b. Because individuals with the dominant type Lu(a–b–) RBCs have normal Lutheran antigens, they do not make anti-Lu3.

In addition to reduced expression of Lutheran antigens, dominant type Lu(a–b–) RBCs also can have reduced expression of CD44 and a weak expression of P1, i, AnWj, MER2, and In^b blood group antigens.

Recessive Type Lu(a–b–)

In some families, the Lu(a–b–) phenotype demonstrates recessive inheritance, the result of having two rare silent alleles *LuLu* at the Lutheran locus. The parents and offspring of these nulls may type Lu(a–b–), but dosage studies and titers show them to carry a single dose of Lu^b.

Unlike the dominant type, people with recessive Lu(a–b–) RBCs truly lack all Lutheran antigens (i.e., they have the null phenotype) and can make an inseparable anti-Lu^{ab} called anti-Lu3. They also have normal antigen expression of P1, i, and the other antigens that are weakened with the dominant type. This distinction emphasizes the importance of testing an antibody against recessive Lu(a–b–) RBCs before defining the specificity phenotypically related to Lutheran.

Different inactivating mutations were recently reported for three individuals with the recessive Lu(a–b–) type.[50] In all three individuals the mutations would result in a truncated glycoprotein that would not be integrated in the RBC surface membrane.

Recessive X-Linked Inhibitor Type

An Lu(a–b–) phenotype in a large Australian family did not fit either the dominant or recessive inheritance patterns. All

Table 8–21 Summary of Lu(a–b–) Phenotypes

MODE OF INHERITANCE	GENE RESPONSIBLE	Lu ANTIGENS	OTHER RBC ANTIGENS AFFECTED	MAKE ANTI-Lu3?
Dominant	*EKLF*[49] Not at *Lu* locus	Extremely weak	Reduced P1, i, In^b, AnWj, MER2, CD44	No
Recessive	*Lu*	None	Not affected	Yes
X-linked	*XS2*	Extremely weak	Not affected	No

Lu(a–b–) family members were male and carried trace amounts of Lu^b detected by adsorption-elution. The pattern of inheritance suggested an X-borne inhibitor to Lutheran. The researchers proposed calling the locus *XS*, *XS1* being the common allele and *XS2* the rare inhibitor that suppresses in a hemizygous state. There have been no other families reported with this rare X-linked Lu(a–b–) phenotype.[9]

Anti-Lu3

Anti-Lu3 is a rare antibody that reacts with all RBCs except Lu(a–b–) RBCs. The antibody looks like inseparable anti-Lu^{ab} and recognizes a common antigen, Lu3, that is present whenever Lu^a or Lu^b is present (much like the Jk3 association with Jk^a and Jk^b). Anti-Lu3 is usually antiglobulin-reactive. This antibody is made only by individuals with the recessive type of Lu(a–b–).

The Diego (010) System

The Diego system is composed of 22 antigens: three sets of independent pairs of antithetical antigens—Di^a/Di^b, Wr^a/Wr^b, and Wu/DISK—and 17 low-prevalence antigens.[3,7] The system was named after the first antibody maker in a Venezuelan family during an investigation of HDFN. The Diego system is designated DI and number 010 by the ISBT.

The Diego antigens are carried on band 3, a major integral RBC membrane glycoprotein with about 1 million copies per RBC. Band 3 is also known as the red cell anion exchanger (AE1) or solute carrier family-4. anion exchanger, member 1 (SLC4A1). The protein crosses the membrane multiple times, and both the amino- and carboxyl-terminal domains are in the cytoplasm. A large *N*-glycan on the fourth external loop carries over half the RBC A, B, H, and I blood group antigens. The long amino-terminal domain of band 3 interacts with ankyrin and protein 4.2 of the membrane skeleton. The gene encoding band 3 and the Diego antigens, *SLC4A1*, consists of 20 exons and is located at chromosome 17q21-q22.

Reported in 1955, anti-Di^a had caused HDFN in a Venezuelan baby. Anti-Di^b was described 2 years later. Di^a is rare in most populations but is polymorphic in people of Mongoloid ancestry. In South American Indians, the prevalence of Di^a can be as high as 54%.[9] Di^a is also present in the North and Central American native populations but is surprisingly rare in Canada and among the Alaskan Inuit.[7] The prevalence of Di^b is generally greater than 99% but is 96% in Native Americans. The Di^a/Di^b polymorphism is located on the last (seventh) extracellular loop of the protein. The Di(a–b–) phenotype has not been reported.

Wr^a, a low-prevalence antigen, and the antithetical, high-prevalence Wr^b are associated with an amino acid substitution on the fourth external loop, close to the insertion point of the protein into the RBC membrane. However, expression of Wr^b requires the presence of both band 3 and a normal GPA of the MNS blood group system. GPA-deficient RBCs are Wr(a–b–).[7]

Localization of Di^a and Di^b antigens to band 3 enabled many low-prevalence antigens to be assigned to the Diego system: Wd^a, Rb^a, WARR, ELO, Wu, Bp^a, Mo^a, Hg^a, Vg^a, Sw^a, BOW, NFLD, Jn^a, KREP, Tr^a, Fr^a, and Sw1.

Diego antigens are expressed on RBCs of newborns. The antigens are resistant to treatment with ficin and papain, DTT, and glycine-acid EDTA, with the exception of Bp^a, which is sensitive to papain.[7,9]

Diego system antibodies are sometimes IgM, but are usually IgG, reactive in the indirect antiglobulin test. Both anti-Di^a and anti-Di^b have caused HTRs and HDFN.[7,9] Anti-Wr^a is a relatively common antibody in donors and patients; some are directly agglutinating, but most require the indirect antiglobulin test to be detected. Anti-Wr^a has caused severe HTRs. Only a few examples of alloanti-Wr^b in individuals with Wr(a–b–) RBCs have been described, so information about clinical significance is insufficient. Autoanti-Wr^b is relatively common in the serum of patients with warm autoimmune hemolytic anemia. Little or no data are available on the clinical significance of antibodies to the low-prevalence Diego antigens, with the exception of anti-ELO, which has caused severe HDFN.[7]

The Yt (011) System

Two antigens make up the Yt system, which was named in 1956 for the first antibody maker and used the last letter "t" in the patient's last name, which was Cartwright.[51] Apparently "why T" became "Yt."[9] Yt^a is the high-prevalence antigen in all populations; Yt^b is the low-prevalence antigen found in about 8% of whites and 21% to 26% of Israelis, but it is not found in Japanese.[7,9] Three phenotypes are observed: the common Yt(a+b–) and Yt(a+b+) and the rare Yt(a–b+). The Yt(a–b–) phenotype has not been reported. The Yt system has the ISBT designation YT and system number 011.

The Yt antigens are antithetical and represent an amino acid substitution on the glycosylphosphatidylinositol (GPI)-linked RBC glycoprotein acetylcholinesterase (AChE). AChE is an important enzyme participating in neurotransmission, but the function of RBC-bound AChE is not known. The gene is located at chromosome 7q22.

Yt antigens are variably sensitive to ficin and papain, are sensitive to DTT, and are resistant to glycine-acid EDTA treatment. The antigens are developed at birth but are expressed more weakly on cord RBCs than on adult RBCs, and are absent from RBCs of people with paroxysmal nocturnal hemoglobinuria (PNH) III.[7]

Anti-Yt^a and anti-Yt^b are IgG and are stimulated by pregnancy or transfusion. Anti-Yt^a is not an uncommon antibody, so it appears that Yt^a is reasonably immunogenic. However, Yt^b appears to be a poor immunogen, as the antibody is rare. Yt antibodies have not caused HDFN. Some examples of anti-Yt^a have been shown to be clinically significant for transfusion while others have not.[7]

The Xg (012) System

Anti-Xg^a was discovered in 1962 in the serum of a multiply transfused man. The antibody detected an antigen with a higher prevalence in females than in males. Family studies were used to confirm the antigen Xg^a expression was controlled by an X-linked gene. The antigen was named after the X chromosome and *g* for "Grand Rapids," where the patient was treated.[9]

The Xg system is designated by the symbol XG and number 012. There are two antigens in the Xg system: Xg^a and CD99. CD99 is also known as 12E7 and MIC2. The gene encoding Xg^a is located on the X chromosome at Xp22.3. The gene responsible for CD99, *MIC2*, is located at Xp22.2. CD99 became part of the Xg system because the *MIC2* and *XG* genes are adjacent and homologous. Xg^a has a phenotypic relationship to CD99: all Xg(a+) individuals have a high expression of CD99 on their RBCs and all Xg(a–) females have a low expression of CD99, but 68% of Xg(a–) males have a high expression and 32% have a low expression of CD99.[7] Both Xg^a and CD99 escape X chromosome inactivation. The Xg glycoprotein crosses the RBC membrane once, with the amino terminus directed externally. There are approximately 9,000 copies of Xg^a per RBC.[9]

The prevalence of Xg^a is 66% in males and 89% in females. Because males have only one X chromosome, Xg(a+) males are hemizygotes. Females, having two X chromosomes, can be homozygotes or heterozygotes. However, homozygosity for the gene does not directly correlate to RBC antigen strength.[52] Cord RBCs express Xg^a weakly. Weak expression of Xg^a is seen on RBCs from some adult females, but weak expression on RBCs from adult males is rare.[9] The antigen is sensitive to ficin and papain but resistant to DTT treatment.

Anti-Xg^a is usually IgG; some examples are naturally occurring. Anti-Xg^a has not been implicated in HDFN or as a cause of HTRs. Two CD99– Japanese individuals have been found with alloanti-CD99.

The Scianna (013) System

The Scianna blood group system, ISBT symbol SC and number 013, currently consists of seven antigens. The system is named after the first antibody maker. In 1962, a new high-prevalence antigen was named Sm; 1 year later, a new low-prevalence antigen, Bu^a was found. After it was confirmed that these two antigens were antithetical, the Scianna blood group system was established in 1974 and the two antigens were renamed Sc1 and Sc2, respectively. The prevalence of Sc2 in Northern Europeans is 1% but is higher in the Mennonite population.

In 1980, an individual in the Marshall Islands in the South Pacific was found with the Sc:–1,–2 phenotype. He had made an antibody to an unknown high-prevalence antibody. The antibody was named anti-Sc3; separable anti-Sc1 and anti-Sc2 were not identified. The very rare Sc:–1,–2,–3 phenotype is the Scianna null type. Three examples of anti-Sc3, nonreactive with Sc:–1,–2, were found to be incompatible with the RBCs of the other anti-Sc3 makers, indicating the existence of additional high-prevalence antigens.[53]

The *SC* gene is located on chromosome 1 at 1p34. The product of the gene is a protein called erythroid membrane-associated protein (ERMAP), which is an RBC adhesion protein. Once location of Scianna to ERMAP was made, other antigens were assigned to the system. The low-prevalence antigen Rd became Sc4. Sc5 (STAR), Sc6 (SCER), and Sc7 (SCAN) are all high-prevalence antigens.[54]

The Scianna antigens are resistant to ficin and papain but are slightly weakened by DTT treatment. The antigens are expressed on cord RBCs.

Alloantibodies to Scianna antigens are rare and little is known about their clinical significance. They are usually IgG and reactive in the antiglobulin test. None have been reported to cause a severe HTR. Only mild HDFN has been reported, except for one severe case for anti-Sc4, for which the baby required exchange transfusion. Autoantibodies to Sc1 and Sc3 have been reported.

The Dombrock (014) System

The Dombrock blood group system, designated by the ISBT with the symbol DO and number 014, was named for the first antibody maker, Mrs. Dombrock, found in 1965. Anti-Do^b, which recognizes the antithetical antigen, was identified in 1973. The prevalence of the three resulting phenotypes, Do(a+b–), Do(a+b+), and Do(a–b+), varies in different populations. In whites, they are 18%, 49%, and 33%, respectively.

The high-prevalence antigens Gy^a and Hy were both described in 1967. RBCs from whites who are Gy(a–) were found to be Hy–, but Hy– RBCs from blacks were weakly Gy(a+). The high-prevalence antigen Jo^a was described in 1972, and the phenotypic relationship to Gy^a and Hy was later shown: Gy(a–) RBCs or Hy– RBCs are also Jo(a–). In 1995, it was reported that Gy(a–) RBCs were also Do(a–b–).[55] The Gy(a–) phenotype is the Dombrock null. Two additional high-prevalence antigens, DOYA and DOMR, were recently added to the system.[3] The Hy– and Jo(a–) phenotypes are not found in whites and are rare in blacks.

The Dombrock antigens are carried on a mono-ADP-ribosyltransferase 4 (ART4) attached to the RBC membrane by a GPI anchor. The gene encoding the Dombrock glycoprotein is located at chromosome 12p12.3.

The Dombrock antigens are resistant to ficin, papain, and glycine-acid EDTA, and are sensitive to 0.2 M DTT treatment. The antigens are present on cord RBCs, but are absent from PNH III RBCs. The Do^a and Do^b antigens are considered to be poor immunogens and the antibodies are rarely found as single specificities; Gy^a, however, is highly immunogenic.[9]

Anti-Do^a and anti-Do^b have caused delayed HTRs but no clinical HDFN. The Dombrock antibodies are usually IgG, reacting optimally with enzyme-treated RBCs. These antibodies are usually weakly reactive and disappear, both factors making them difficult to identify.

The Colton (015) System

The Colton blood group system, ISBT symbol CO and number 015, consists of four antigens. The system was named in 1967 for the first antibody maker; it should have been named Calton, but the handwriting on the tube was misread![9] The high- and low-prevalence antithetical antigens are Co^a and Co^b, respectively. The Co^b antigen is present in about 10% of most populations.[9] The third antigen, Co3, is present on all RBCs except those of the very rare Co(a–b–) phenotype. Co4, a high-prevalence antigen, has been identified on RBCs from two individuals with the Co(a–b–) phenotype.[3]

The Colton antigens are carried on an integral membrane protein, aquaporin 1 (AQP1), which accounts for 80% of water readsorption in the kidneys.[9] The glycoprotein crosses the RBC membrane multiple times. The gene (*AQP1*) is located at chromosome 7p14. The Colton antigens are expressed on RBCs of newborns and are resistant to treatment with ficin and papain, chloroquine, and DTT.

Antibodies are usually IgG and are enhanced with enzyme-treated RBCs. Anti-Co^a is often seen as a single specificity and has been reported to cause HTRs and HDFN.[56] Anti-Co^b appears more often with other specificities but has also caused HTRs and mild HDFN.[9] Anti-Co3, which reacts with all Co(a+) and Co(b+) RBCs, has been reported to cause severe HDFN.[1]

The Landsteiner-Wiener (016) System

The Landsteiner-Wiener blood group system, ISBT symbol LW and number 016, had its origins along with the discovery of the D antigen of the Rh blood group system. In 1940, Landsteiner and Wiener reported that an antibody produced in rabbits (and later, guinea pigs) after injection with RBCs of rhesus monkeys reacted with 85% of human RBCs.[57] This antibody was called anti-Rh for anti-rhesus. The reactivity of anti-Rh was similar to the human antibody reported by Levine and Stetson in 1939 in a woman who delivered a stillborn infant and had a transfusion reaction to the blood donated by her husband.[58] Both sera identified the same population of Rh+ and Rh– RBCs. The two antibodies were later shown to be different in several studies; in the 1960s, the human anti-Rh was renamed anti-D (but called anti-Rh_o by some workers) and the rabbit anti-Rh was called anti-LW in honor of Landsteiner and Wiener. Examples of human anti-LW were subsequently described.

There are three LW antigens: LW^a, LW^{ab}, and LW^b. The first two, LW^a and LW^{ab}, are common, high-prevalence antigens, and LW^b is of low prevalence, found in less than 1% of most Europeans but in 6% of Finns.[9] LW^{ab} was originally defined by the antibody made by an individual with an inherited LW(a–b–) phenotype. Terminology for LW antigens has evolved as more information became available. The ISBT has used LW5, LW6, and LW7 as the antigen numbers for LW^a, LW^{ab}, and LW^b, respectively, to prevent confusion with obsolete terminology that used LW1-LW4 to designate phenotypes. The null phenotype is LW(a–b–); in one individual who made anti-LW^{ab} (Mrs. Big), this phenotype resulted from a 10 base pair deletion in exon 1 of an LW^a gene, which introduced a premature stop codon. Rh_{null} RBCs also type LW(a–b–) and are considered to be the only true LW– RBCs because they fail to elicit the formation of anti-LW in animals, whereas injection of Mrs. Big's LW(a–b–) RBCs into guinea pigs caused the formation of anti-LW. The LW phenotypes are shown in Table 8–22.

As was shown by the similarity in reactivity of the original animal anti-Rh and the human anti-Rh (later called anti-D), there is a phenotypic relationship between the D antigen and anti-LW. Anti-LW usually reacts strongly with D+ RBCs, weakly (and sometimes not at all) with D– RBCs from adults, and not at all with Rh_{null} RBCs. A weak anti-LW may react only with D+ RBCs and may appear to be anti-D unless enhancement techniques are used. This is because there are more LW antigen sites on D+ RBCs from adults than on D– RBCs.[9] However, anti-LW reacts equally well with cord RBCs regardless of their D type. Distinguishing anti-LW

Table 8–22 **LW Phenotypes**

PHENOTYPE	REACTIVITY WITH			PREVALENCE	
	Anti-LW^a	Anti-LW^b	Anti-LW^{ab}	Most Europeans	Finns
LW(a+b–)	+	–	+	97%	93.9%
LW(a+b+)	+	+	+	3%	6%
LW(a–b+)	–	+	+	Rare	0.1%
LW(a–b–) Big	–	–	–		
LW(a–b–) Rh_{null}	–	–	–		

from anti-D is most easily accomplished by testing DTT-treated D+ RBCs: the D antigen is not denatured by DTT, so anti-D would still be detected; however, LW antigen is destroyed by DTT, so anti-LW would no longer react. LW antigens are resistant to treatment of RBCs with enzymes and glycine-acid EDTA.

The structure that carries the LW antigens is a glycoprotein known as intracellular adhesion molecule 4 (ICAM-4), a member of the immunoglobulin superfamily. The LW glycoprotein is part of the band 3/Rh macrocomplex[59]; Rh_{null} RBCs lack the LW glycoprotein. The *LW* gene is located on chromosome 19 at 19p13.3.

LW antigens may be depressed during pregnancy and in some diseases, such as lymphoma and leukemia.[9] Autoanti-LW made by these patients can appear to be an alloantibody; as the antigen strength returns the antibody diminishes. Autoanti-LW is also common in serum from patients with warm autoimmune hemolytic anemia. No anti-LW has been shown to cause serious HDFN or transfusion reactions. Many patients with anti-LW have successfully been transfused with D– RBCs. Only two examples of alloanti-LW^{ab} have been described, the antibodies of the only two known propositi with an inherited LW(a–b–) phenotype.[7]

The Chido/Rodgers (017) System

The Chido/Rodgers blood group system, designated by the ISBT with symbol CH/RG and number 017, was named after the first two antibody producers, Ch for Chido and Rg for Rodgers. These two antibodies were described in 1967 and 1976, respectively. Serologically, they were both characterized as nebulous because antigen strength on different samples of RBCs was variable.[1] It was also appreciated that both anti-Ch and anti-Rg could be neutralized by plasma.

Ch and Rg antigens are not intrinsic to the RBC membrane. Rather, they are on the fourth component of complement (C4), and are adsorbed onto RBCs from plasma.[1] The C4 glycoprotein has two isoforms: C4B carries the Ch antigens and C4A expresses Rg antigens. Genes at two closely linked loci located at chromosome 6p21.3 encode these isoforms. The Chido/Rodgers system consists of nine antigens: Ch1 to Ch6, Rg1, and Rg2 are all high prevalence; WH has a prevalence of about 15%.[9] Differentiation of the determinants is not made for routine serology.

Ch is present in 96% to 98% of most populations.[1,9] Rg is present in 97% to 98% of most populations.[1,9] The antigens are destroyed by ficin and papain but are resistant to treatment with DTT and glycine-acid EDTA.[9]

Anti-Ch and anti-Rg are usually IgG and react weakly, often to moderate or high titration endpoints. Neutralization of anti-Ch and anti-Rg with pooled plasma is often used as part of the identification of these antibodies in a patient's serum. Anti-Ch and anti-Rg are clinically insignificant for transfusion.[1]

The Gerbich (020) System

The Gerbich blood group system was named in 1960 after Mrs. Gerbich, the first antibody producer. Gerbich became a system in 1990, designated by the ISBT as GE and number 020. There are currently six high-prevalence Gerbich antigens (Ge2, Ge3, Ge4, GEPL, GEAT, and GETI) and five low-prevalence antigens (Wb, Ls^a, An^a, Dh^a, and GEIS). The antigens are carried on sialoglycoprotein structures GPC and GPD. The glycoproteins help to maintain the RBC membrane integrity through interaction with protein band 4.1, and because they are rich in sialic acid, they contribute to the net negative charge of the RBC membrane (as do GPA and GPB of the MNS system). There are about 135,000 copies of GPC and 50,000 copies of GPD per RBC.[9] GPC and GPD are both encoded by the *GYPC* gene, located on chromosome 2 at 2q14.3.

There are three Gerbich-negative phenotypes in which the RBCs lack one or more of the high-prevalence antigens: Ge:–2,3,4 (Yus type), Ge:–2,–3,4 (Gerbich type), and Ge:–2,–3,–4 (Leach type). The Leach type is the Gerbich null phenotype. These are summarized in Table 8–23. Outside of Papua, New Guinea, the Gerbich-negative phenotypes are very rare, but they have been found in diverse populations. The Yus phenotype has been found in Mexicans, Israelis, and others but has not been found in Papua, New Guinea and other Melanesians.[9] The Gerbich phenotype is polymorphic in certain areas of Papua, New Guinea and has also been found among Europeans, Africans, Native Americans, Japanese, and Polynesians.[9]

Gerbich antigens are expressed at birth. RBCs of the Gerbich or Leach phenotypes have weak expression of Kell blood group antigens, and some anti-Vel fail to react with Ge:–2,–3,4 RBCs.[9] Gerbich antigens are resistant to treatment with DTT and glycine-acid EDTA; Ge2 and Ge4 are ficin and papain sensitive, but Ge3 is ficin resistant.

Some Gerbich antibodies may be IgM, but most are IgG. Gerbich antibodies are sometimes clinically significant for transfusion and sometimes not. Gerbich antibodies can be eluted from DAT+ cord bloods, but only three cases of serious HDFN due to anti-Ge3 have been reported, and two were children of the same mother.[60,61] In these cases, the severe anemia was late onset, after birth, associated with inhibition of erythroid cell growth; in one case there was also early onset of hemolysis.

Anti-Ge2 is the most common of the Gerbich antibodies.[7] It is the antibody made by the Ge:–2,3,4 phenotype individuals, but it is also the more common antibody made by the Ge:–2,–3,4 phenotype and Ge:–2,–3,–4 phenotype individuals. Anti-Ge3 is less frequently made by the Ge:–2,–3,4 and Ge:–2,–3,–4 phenotype individuals. Anti-Ge4 is a very rare antibody.

Table 8–23 Gerbich-Negative Phenotypes

PHENOTYPE	TYPE	ANTIBODY
Ge: –2, 3, 4	Yus	Anti-Ge2
Ge: –2, –3, 4	Gerbich	Anti-Ge2 or anti-Ge3
Ge: –2, –3, –4	Leach type	Anti-Ge2 or anti-Ge3

The Cromer (021) System

In 1965, an antibody was found in a black prenatal patient, Mrs. Cromer, that reacted with all RBCs except her own and two siblings. It was originally thought her antibody recognized an antigen antithetical to Go^a of the Rh blood group system. The antibody was named anti-Cr^a in 1975.[7,9]

The antigens of the Cromer system are carried on decay accelerating factor (DAF, CD55), a complement regulatory protein. The *CD55* gene is located at chromosome 1q32. The glycoprotein encoded by the gene is attached to the RBC membrane through a GPI linkage. PNH III RBCs are deficient in DAF so they also lack Cromer antigens.

The Cromer system has 16 antigens: 13 high-prevalence antigens and 3 low-prevalence antigens.[62,63] All these antigens are absent from Inab phenotype RBCs, the Cromer null phenotype, which is very rare. The Cr(a–) phenotype is typically found in blacks and is not found in whites. Three Cromer antigens, Tc^a, Tc^b, and Tc^c, are antithetical; Tc^a is the high-prevalence antigen and the other two are low prevalence. The Dr^a antigen is of high prevalence; Dr(a–) RBCs have weakened expression of all other high-prevalence Cromer antigens due to a markedly reduced copy number of DAF.

Cromer system antigens are resistant to treatment with ficin and papain but are destroyed by α-chymotrypsin, which is used to distinguish specificities in this system from other blood group antibodies. Cromer antigens are weakened with DTT treatment and are resistant to glycine-acid EDTA.

Antibodies in the Cromer system are usually IgG, but do not cause HDFN. DAF is strongly expressed on placental tissue and will adsorb Cromer antibodies.[9,63] Anti-Cr^a and anti-Tc^a have been implicated in HTRs, but other examples of these specificities have not caused clinical reactions after transfusion of incompatible units.[62] Anti-IFC is the antibody made by individuals with the Cromer null Inab phenotype.

The Knops (022) System

There are nine antigens in the Knops blood group system, designated by the ISBT as KN and number 022. The system was established when the antigens were shown to be located on complement receptor 1 (CR1) and was named after Mrs. Knops, the first antibody maker. The gene is located at chromosome 1q32.

With the exception of the low-prevalence antigens Kn^b and McC^b, the Knops antigens have a prevalence of more than 90% in most populations; however, ethnic differences exist. Sl^a is present on RBCs of only about 60% of African Americans. Among West Africans, Sl^a has a prevalence of 30% to 38%, and KCAM has a prevalence of only 20%.[64] The antithetical pairs of antigens are Kn^a and Kn^b, McC^a and McC^b, and Sl^a and Vil. Knops antigens are weakly expressed on cord RBCs and weaken upon storage of adult RBCs (e.g., older units of blood). The antigens are weakened by treatment with ficin and papain and are destroyed by DTT; the antigens are resistant to glycine-acid EDTA.[9]

Serologically, these antigens have been grouped together because their corresponding antibodies demonstrate variable reactions, are not neutralized by pooled normal serum (unlike anti-Ch and anti-Rg), and are difficult to adsorb and elute.[64] Antibody reactivity is enhanced with longer incubation (e.g., 1 hour, at 37°C). Weak and variable reactivity is due to variable expression of CR1 on different samples of RBCs. The "Helgeson phenotype" represents the serologic null phenotype for the Knops blood group; these RBCs type Kn(a–b–), McC(a–), Sl(a–), and Yk(a–) because of the low copy number of CR1, but they are not truly devoid of Knops antigens.[9,64]

The antibodies are usually IgG, reactive in the antiglobulin test, but are clinically insignificant for both transfusion and HDFN. Of the Knops antibodies, anti-Kn^a is frequently found in multiply transfused individuals and multispecific sera; anti-Sl^a is more frequently found in blacks.

CR1 binds the complement component fragments C3b and C4b and processes immune complexes for transportation to the liver and spleen and subsequent clearance from the circulation.[7,64] CR1 has also been identified as a receptor for several pathogenic organisms.

The Indian (023) System

The Indian blood group system was named because the first In(a+) individuals were from India. There are now four antigens in the system, designated IN and number 023 by the ISBT. The antigen In^a was reported in 1973 and is present on RBCs of 4% of Indians, 11% of Iranians, and 12% of Arabs.[9] In^b is the antithetical high-prevalence antigen. These and two other high-prevalence antigens are located on CD44, an adhesion molecule. The gene encoding CD44 is located at chromosome 11p13. The extremely rare In(a–b–) phenotype has been found in only one individual who presented with congenital dyserythropoietic anemia and whose RBCs also typed Co(a–b–).[65]

CD44 is reduced on RBCs of dominant type Lu(a–b–) individuals. In^a and In^b are weakly expressed on cord RBCs and are sensitive to treatment with ficin, papain, and DTT but are resistant to glycine-acid EDTA.[9]

Antibodies are usually IgG and reactive in the antiglobulin test and they do not bind complement. Positive DATs but no clinical HDFN have been reported for anti-In^a and anti-In^b; decreased cell survival with anti-In^a and an immediate HTR due to anti-In^b have been reported.[9]

The Ok (024) System

Currently, there are three high-prevalence antigens in the Ok system, designated by the ISBT symbol OK and number 024. Anti-Ok^a was identified in 1979 and was named after the antibody maker, Mrs. Kobutso. Because *Ko* was already in use, the first two letters were switched to *Ok*.[2] Her parents were cousins from a small Japanese island. Two of three siblings of the proposita were also Ok(a–).[7] RBCs from 400 individuals from the same island were all Ok(a+).[1] Two additional antigens, OKGV and OKVM, were recently added to the system.

The OK antigens are carried on CD147, a member of the immunoglobulin superfamily that mainly functions as receptors and adhesion molecules. The gene locus is at chromosome 19p13.3.

Ok^a is well developed on RBCs from newborns and is resistant to treatment with ficin and papain, DTT, and glycine-acid EDTA.[9]

The original anti-Ok^a was IgG, reactive in the antiglobulin test. At least one other example of the antibody has been found. The antibody caused reduced survival of ^{51}Cr-labeled Ok(a+) RBCs injected into the original antibody maker.[1] Anti-Ok^a has not been reported to cause HDFN.

The Raph (025) System

The only antigen in the Raph system is MER2, which was originally defined by two monoclonal antibodies; it has since been recognized by human polyclonal antibodies. The antigen name is derived from *m*onoclonal, and *E*leanor *R*oosevelt, the laboratory where the antibody was produced. When MER2 was raised to system status, the system was named Raph for the first patient to make the alloanti-MER2. MER2 is encoded by a gene located at chromosome 11p15.

Three examples of alloanti-MER2 were found in Jews originating from India and living in Israel; two were related. All three had end-stage renal disease. A fourth example of anti-MER2 was found in a healthy Turkish blood donor. Blood samples from these four individuals were studied. It was shown that MER2 is located on CD151, a tetraspanin, which appears to be essential for the assembly of basement membranes in the kidney and skin.[66]

The polymorphism identified by the monoclonal antibodies indicated that 8% of the English blood donor population is MER2–. Subsequent studies suggest the antigen-negative status represents the low end of antigen expression on RBCs.[66] MER2 is abundant on platelets and is expressed on erythroid precursors of individuals with either MER2+ or MER2– RBCs. MER2 expression decreases over time with increasing maturation of erythroid cells.[66] It has been suggested that most people whose RBCs type MER2– have MER2 expressed on other cells and tissues and will not make anti-MER2, and those individuals who have made alloanti-MER2 are true MER2–. Those individuals who have made anti-MER2 showed mutations in *CD151*. In the patients with renal disease, a truncated CD151 protein would be predicted, but in patients without renal disease, mutations would result in an altered CD151 that appears to be present and functional at the cell surface.[66,67]

The antigen is resistant to treatment with ficin and papain but is sensitive to treatment with trypsin, α-chymotrypsin, pronase, and AET.

Little is known about the clinical significance of anti-MER2, but one patient with the antibody showed signs of a transfusion reaction after transfusion with 3 units. As 8% of the population is expected to be MER2–, it would be prudent to transfuse with crossmatch compatible units.[67]

The John Milton Hagen (026) System

JMH is a high-prevalence antigen. Numerous examples of anti-JMH have been seen, especially in patients 50 years and older. In 1978, a large number of samples with this antibody were characterized and the antibody was named anti-JMH for the first antibody maker, John Milton Hagen. The system (ISBT symbol JMH and number 026) was established after it was shown that the JMH protein is the GPI-linked glycoprotein CD108 and the gene *SEMA7A* was cloned. The gene is located at chromosome 15q24.1.

Five other antigens were recently added to the system, JMH2 through JMH6; these are JMH variants associated with amino acid substitutions in the protein.[68] JMH1 represents the antigen recognized by antibodies made by individuals lacking the JMH protein.

Most examples of anti-JMH are not found in patients who lack the JMH protein or who have one of the variant JMH phenotypes. Rather, the patient's JMH– status is acquired and can be transient.[1] It is widely accepted that JMH levels decline during the later years of life, sometimes to the point of not being detected serologically.[1] Once JMH expression is reduced, anti-JMH can be made. In some cases with anti-JMH, the DAT is positive and some JMH is detected on the patient's RBCs. This autoanti-JMH with a positive DAT has never been associated with autoimmune RBC destruction.[1]

JMH is weakly expressed on cord RBCs and is destroyed by treating RBCs with ficin and papain, and DTT; the antigen is resistant to treatment with glycine-acid EDTA.[9]

Anti-JMH is usually IgG (predominantly IgG4 in acquired JMH-negative people).[9] The antibodies are often high titer but weakly reactive, even when tested without dilution, and they are not neutralized with pooled plasma. JMH antibodies are generally considered clinically insignificant. Rare examples of alloanti-JMH in individuals whose RBCs express variant forms of CD108 may be clinically significant.[1]

The Gill (029) System

Anti-GIL was first identified in 1980, but the antigen was not raised to system status until 2002 when it was shown that GIL is genetically discrete from all other blood group systems. The ISBT Gill system symbol is GIL and number 029. There is only one antigen, GIL.

This antigen is found on the glycerol transporter aquaporin 3 (AQP3), a member of the major intrinsic protein family of water channels. *AQP3* is located at chromosome 9p13. The GIL$_{\text{null}}$ phenotype results from a frameshift and a premature stop codon.

Reactivity with anti-GIL is enhanced with ficin and papain treatment of RBCs; the antigen is resistant to DTT and glycine-acid EDTA treatment.

RBCs of two babies born to mothers with anti-GIL have had a positive DAT but no clinical HDFN.[7] One example of anti-GIL was found following a hemolytic transfusion reaction.

The RH-Associated Glycoprotein (030) System

Rh-associated glycoprotein is the newest blood group system (IBST symbol RHAG and number 030).[3] The Rh-associated glycoprotein (RhAG) does not have Rh blood group antigens; however, its presence in a complex with the Rh proteins is essential for Rh antigen expression. Absence of RhAG due to inactivating mutations in *RHAG* results in the Rh_{null} phenotype; some missense mutations in RHAG result in the Rh_{mod} phenotype.[69] Unlike the RhD and RhCcEe proteins, RhAG is glycosylated on the first extracellular loop. RhAG is encoded by *RHAG*, located at chromosome 6p11-21.

Two antigens have been definitively assigned to the RHAG system: Duclos (RHAG1), previously 901013 in the high-prevalence series, and Ol^a (RHAG2), previously 700043 in the low-prevalence series. Two antigens, DSLK (for Duclos-like) and RHAG4, have been provisionally assigned to the system.

Miscellaneous Antigens

Vel

Vel, a high-prevalence antigen, is in the Vel collection, along with another high-prevalence antigen ABTI. Anti-Vel was first described in 1952 and was named after the first antibody maker. Anti-Vel is characterized by its ability to activate complement and cause in vitro and in vivo hemolysis. The antibody is most often IgG but can be IgM, and it has caused severe, immediate HTRs.[1,7] Anti-Vel has also caused one case of severe HDFN in which the mother had previously been transfused.[9]

Vel antigen expression is weak on cord RBCs and can be variable on RBCs of different adult individuals. RBCs with weak expression of Vel can be mistyped as Vel–. This is one of the reasons why anti-Vel can be a difficult antibody to work with and identify. Reactivity with anti-Vel can be enhanced with enzyme-treated RBCs. The antigen is also resistant to glycine-acid EDTA and DTT treatment, though some examples of anti-Vel do not react with DTT-treated RBCs.[70]

At^a

Anti-At^a was first described in 1967 in the serum of a black woman named Mrs. Augustine.[1] At^a is a high-prevalence antigen, and all At(a–) individuals have been black. The antigen is fully developed at birth and is resistant to treatment with ficin and papain, DTT, and glycine-acid EDTA. The antibody is usually IgG, reactive in the antiglobulin test. Anti-At^a has caused severe HTRs and one reported mild case of HDFN.[7,9]

Jr^a

Jr^a is a high-prevalence antigen in most populations; the Jr(a–) phenotype is found more commonly in Japanese. The first anti-Jr^a was described in 1970; several examples have since been found. The antigen is fully developed at birth and is resistant to treatment with ficin and papain, DTT, and glycine-acid EDTA.

Anti-Jr^a is usually IgG. Clinical significance of anti-Jr^a is not well established since it is a rare antibody. One fatal case of HDFN was recently reported; in this case, the mother had previously been massively transfused, whereas in previous cases of no to mild HDFN, the mothers had not been transfused.[71] Some patients with anti-Jr^a have received Jr(a+) incompatible units for transfusion and have had no ill effect, but in other cases, incompatible transfusions have resulted in HTRs.[72]

Sd^a

The Sd^a antigen is a high-prevalence antigen named for Sid, who was the head of the maintenance department at the Lister Institute in London. His RBCs had been used for many years as a panel donor, and they reacted strongly with examples of a new antibody. The soluble form of Sd^a is Tamm-Horsfall glycoprotein found in urine. The antigen is not expressed on RBCs of newborns but is in their saliva, urine, and meconium. The strength of Sd^a on adult RBCs varies and is markedly reduced in pregnancy. The antigen is found on 91% of RBC samples, and Sd^a substance is found in 96% of urine samples. Only 4% of people are Sd(a–).[9] Strong examples of Sd^a are noted as Sd(a++).

Anti-Sd^a can naturally occur (i.e., without known stimulation by transfusion or pregnancy) in the sera of individuals who are Sd(a–). Anti-Sd^a is usually an IgM agglutinin that is reactive at room temperature, but it can be detected in the indirect antiglobulin test and does not react with cord RBCs. Reactivity is described as small, refractile (shiny) agglutinates in a sea of free RBCs. Because the soluble antigen is present in urine of Sd(a+) individuals, neutralization of the refractile agglutinates by urine is a technique used to identify anti-Sd^a. The Sd^a antigen is resistant to treatment with ficin, papain, DTT, and glycine-acid EDTA. Reactivity of the antibody is enhanced with enzyme-treated RBCs. Anti-Sd^a is generally considered clinically insignificant for transfusion, though there are two reports of transfusion reactions associated with the transfusion of Sd(a++) RBCs.

Applications to Routine Blood Banking

The major blood group systems outside of ABO and Rh become important only after patients develop unexpected antibodies. Then a fundamental knowledge of antibody characteristics, clinical significance, and antigen frequency is needed to help confirm antibody specificity and to select appropriate units for transfusion.

Only a few antibody specificities are commonly seen: M, P1, and I antibodies react at room temperature and are considered clinically insignificant; K, S, s, Fy^a, Fy^b, Jk^a, and Jk^b antibodies react in the antiglobulin phase and are clinically significant. These and selected others are summarized in Table 8–24.

Not all antibody problems are easily solved; panel reactions are sometimes inconclusive. As described in this chapter, the existence of silent, regulator, and inhibitor genes can affect antigen expression. Hopefully the reader will find that the information in this chapter provides a starting point for serologic problem-solving. For further detailed information about the RBC antigens and antibodies described here, see Issitt and Anstee[1] and Daniels.[7] Resolution of antibody problems involving the unusual specificities described here may require the assistance of an immunohematology reference laboratory.

Table 8–24 **Summary of Antibody Characteristics**

ANTIBODY	REACTIVITY			ENZYMES	BIND COMPLEMENT	IN VITRO HEMOLYSIS	HTR	HDFN	COMPATIBLE IN U.S. POPULATION (%)
	≤RT	37	AHG						
M*	Most	Few	Few	Destroy	No	No	Few	Mild–severe	22
N*	Most	Few	Few	Destroy	No	No	Rare	Moderate	2B
S	Some	Some	Most	Variable effect	Some	No	Yes	Mild	45 W 69 B
s	Few	Few	Most	Variable effect	Rare	No	Yes	Mild–severe	11 W 3 B
U	Rare	Some	Most	No change	No	No	Yes	Mild–severe	< 1 B
P1*	Most	Some	Rare	Enhance	Rare	Rare	Rare	No	21
PP1P^k†	Most	Some	Some	Enhance	Most	Most	Yes	Mild	< 0.1
P‡	Most	Some	Some	Enhance	Most	Some	Yes	Mild–severe	< 0.1
I*	Most	Few	Few	Enhance	Most	Few	Rare	No	See text
i*	Most	Few	Few	Enhance	Most	Few	No	Mild	See text
K	Some	Some	Most	No change	Rare	No	Yes	Mild–severe	91
k	Few	Few	Most	No change	No	No	Yes	Mild	0.2
Kp^a	Some	Some	Most	No change	No	No	Yes	Mild	99.7
Kp^b	Few	Few	Most	No change	No	No	Yes	Mild	< 0.1
Js^a	Few	Few	Most	No change	No	No	Yes	Moderate	100 W 80 B
Js^b	No	No	Most	No change	No	No	Yes	Mild–moderate	1 B
Fy^a	Rare	Rare	Most	Destroy	Rare	No	Yes	Mild–severe	34 W
Fy^b	Rare	Rare	Most	Destroy	Rare	No	Yes	Mild	17 W 77 B
Jk^a‡	Few	Few	Most	Enhance	Yes§	Some	Yes	Mild	23
Jk^b‡	Few	Few	Most	Enhance	Yes§	Some	Yes	Mild	28 W 57 B
Lu^a	Most	Few	Few	Variable effect	Some	No	No	Mild	92
Lu^b	Few	Few	Most	Variable effect	Some	No	Yes	Mild	0.15

B = blacks; HDFN = hemolytic disease of the newborn; HTR = hemolytic transfusion reactions; ≤ RT = room temperature or colder; W = whites.

*Usually clinically insignificant

†Potent hemolysins may be associated with early abortions

‡Associated with severe delayed HTR

§Provided IgM is present

SUMMARY CHART

Blood Group Terminology

- ✓ The ISBT terminology for RBC surface antigens provides a standardized numeric system for naming authenticated antigens that is suitable for electronic data processing equipment. This terminology was not intended to replace conventional terminology.
- ✓ In the ISBT classification, RBC antigens are assigned a six-digit identification number: The first three digits represent the system, collection, or series, and the second three digits identify the antigen. All antigens are catalogued into one of the following four groups:
  - A blood group system if controlled by a single gene locus, or by two or more closely linked genes
  - A collection if shown to share a biochemical, serologic, or genetic relationship
  - The high-prevalence series (901) if found in more than 90% of most populations
  - The low-prevalence series (700) if found in less than 1% of most populations

Lewis Blood Group

- ✓ Lewis blood group antigens are not synthesized by the RBCs. These antigens are adsorbed from plasma onto the RBC membrane.
- ✓ The *Le* gene codes for L-fucosyltransferase, which adds L-fucose to type 1 chains.
- ✓ The *Le* gene is needed for the expression of Le^a substance, and *Le* and *Se* genes are needed to form Le^b substance.
- ✓ The *lele* genotype is more common among blacks than among whites and results in the Le(a–b–) phenotype.
- ✓ Lewis antigens are poorly expressed at birth.
- ✓ Lewis antibodies are generally IgM (naturally occurring) made by Le(a–b–) individuals.
- ✓ Lewis antibodies are frequently encountered in pregnant women.
- ✓ Lewis antibodies are not considered significant for transfusion medicine.

The P Blood Group

- ✓ The P blood group consists of the biochemically related antigens P, P1, P^k and LKE.
- ✓ P1 antigen expression is variable; P1 antigen is poorly developed at birth.
- ✓ Anti-P1 is a common naturally occurring IgM antibody in the sera of P1– individuals; it is usually a weak, cold-reactive saline agglutinin and can be neutralized with soluble P1 substance found in hydatid cyst fluid.
- ✓ Anti-PP1P^k is produced by the rare p individuals early in life without RBC sensitization and reacts with all RBCs except those of other p individuals. Antibodies may be a mixture of IgM and IgG, efficiently bind complement, may demonstrate in vitro hemolysis, and can cause severe HTRs. Anti-PP1P^k is associated with spontaneous abortions.
- ✓ Alloanti-P is found as a naturally occurring alloantibody in the sera of P^k individuals and is clinically significant.
- ✓ Autoanti-P is most often the specificity associated with the cold-reactive IgG autoantibody in patients with paroxysmal cold hemoglobinuria (PCH).
- ✓ The autoanti-P of PCH usually does not react by routine tests but is demonstrable as a biphasic hemolysin only in the Donath-Landsteiner test.

The I and i Antigens

- ✓ I and i antigens are not antithetical; they have a reciprocal relationship.
- ✓ Most adult RBCs are rich in I and have only trace amounts of i antigen.
- ✓ At birth, infant RBCs are rich in i; I is almost undetectable; over the next 18 months of development, the infant's RBCs will convert from i to I antigen.
- ✓ Anti-I is typically a benign, weak, naturally occurring, saline-reactive IgM autoagglutinin, usually detectable only at 4°C.
- ✓ Pathogenic anti-I is typically a strong cold autoagglutinin that demonstrates high-titer reactivity at 4°C and reacts over a wide thermal range (up to 30°–32°C).
- ✓ Patients with *M. pneumoniae* infections may develop strong cold agglutinins with autoanti-I specificity.
- ✓ Anti-i is a rare IgM agglutinin that reacts optimally at 4°C; potent examples may be associated with infectious mononucleosis.

The MNS Blood Group System

- ✓ Anti-M and anti-N are cold-reactive saline agglutinins that do not bind complement or react with enzyme-treated cells; both anti-M and anti-N may demonstrate dosage.
- ✓ Anti-S and anti-s are IgG antibodies, reactive at 37°C and the antiglobulin phase. They may bind complement and have been associated with HDFN and HTRs.
- ✓ The S–s–U– phenotype is found in blacks.
- ✓ Anti-U is usually an IgG antibody and has been associated with HTRs and HDFN.

The Kell Blood Group System

- ✓ The Kell blood group antigens are well developed at birth and are not destroyed by enzymes.
- ✓ The Kell blood group antigens are destroyed by DTT, ZZAP, and glycine-acid EDTA.
- ✓ Excluding ABO, the K antigen is rated second only to D antigen in immunogenicity.

SUMMARY CHART—cont'd

- ✓ The k antigen is high prevalence.
- ✓ Anti-K is usually an IgG antibody reactive in the antiglobulin phase and is made in response to pregnancy or transfusion of RBCs; it has been implicated in severe HTRs and HDFN.
- ✓ The McLeod phenotype, affecting only males, is described as a rare phenotype with decreased Kell system antigen expression. The McLeod syndrome includes the clinical manifestations of abnormal RBC morphology, compensated hemolytic anemia, and neurological and muscular abnormalities. Some males with the McLeod phenotype also have the X-linked chronic granulomatous disease.

The Duffy Blood Group System

- ✓ Fy^a and Fy^b antigens are destroyed by enzymes and ZZAP; they are well developed at birth. The Fy(a–b–) phenotype is prevalent in blacks but virtually nonexistent in whites.
- ✓ Fy(a–b–) RBCs resist infection by the malaria organism *P. vivax*.
- ✓ Anti-Fy^a and anti-Fy^b are usually IgG antibodies and react optimally at the antiglobulin phase of testing; both antibodies have been implicated in delayed HTRs and HDFN.

The Kidd Blood Group System

- ✓ Anti-Jk^a and anti-Jk^b may demonstrate dosage, are often weak, and found in combination with other antibodies; both are typically IgG and reactive in the antiglobulin test.
- ✓ Kidd system antibodies may bind complement and are made in response to foreign RBC exposure during pregnancy or transfusion.
- ✓ Kidd system antibodies are a common cause of delayed HTRs.
- ✓ Kidd system antibody reactivity is enhanced with enzymes, LISS, and PEG.

The Lutheran Blood Group System

- ✓ Lu^a and Lu^b are antigens produced by codominant alleles; they are poorly developed at birth.
- ✓ Anti-Lu^a may be a naturally occurring saline agglutinin that reacts optimally at room temperature.
- ✓ Anti-Lu^b is usually an IgG antibody reactive at the antiglobulin phase; it is usually produced in response to foreign RBC exposure during pregnancy or transfusion.
- ✓ The Lu(a–b–) phenotype is rare and may result from three different genetic backgrounds; only individuals with the recessive type Lu(a–b–) can make anti-Lu3.

Other Blood Groups and Antigens

- ✓ The Diego system antigens are located on a major RBC protein, band 3, also known as the RBC anion exchanger (AE1).
- ✓ Anti-Di^a, anti-Di^b, and anti-Wr^a are generally considered to be clinically significant; all have caused severe HTRs and HDFN. Anti-Wr^a is a relatively common antibody.
- ✓ Wr^b expression requires the presence of a normal GPA (MNS system); alloanti-Wr^b is extremely rare.
- ✓ Anti-Yt^a is a fairly common antibody to a high-prevalence antigen that is sometimes clinically significant and sometimes insignificant.
- ✓ The Xg^a antigen is found on the short arm of the X chromosome and is of higher prevalence in females (89%) than in males (66%). Although it is usually IgG, anti-Xg^a has not been implicated in HDFN or as a cause of HTRs.
- ✓ Antibodies to Scianna system antigens are rare and little is known about their clinical significance. The rare null phenotype, Sc:–1,–2,–3, has been observed in the Marshall Islands and New Guinea.
- ✓ In addition to the Do^a and Do^b antigens, the Gy^a, Hy, Jo^a antigens are assigned to the Dombrock system. Anti-Do^a and anti-Do^b have caused HTRs but no clinical HDFN; these antibodies are usually weak and difficult to identify.
- ✓ The Colton system is composed of the antithetical Co^a and Co^b antigens as well as the high-prevalence Co3 antigen; the antigens are carried on aquaporin 1, a red cell water channel. The Colton antibodies have caused HTRs and HDFN.
- ✓ LW has a phenotypic relationship with the D antigen; Rh$_{null}$ RBCs type LW(a–b–).
- ✓ Anti-LW reacts strongly with D+ RBCs and can look like anti-D. DTT treatment of test RBCs will distinguish between these two antibodies because the LW antigen is denatured by DTT, but the D antigen is not. In other words, anti-LW does not react with DTT-treated D+ RBCs but anti-D does.
- ✓ The antigens in the Chido/Rodgers system are located on the complement fragments C4B and C4A, respectively, that are adsorbed onto RBCs from plasma.
- ✓ The clinically insignificant anti-Ch and anti-Rg react weakly, often to moderate or high-titer endpoints in the antiglobulin test and may be tentatively identified by plasma inhibition methods.
- ✓ Gerbich-negative phenotypes are very rare outside of Papua, New Guinea.

Continued

SUMMARY CHART—cont'd

- ✓ Gerbich antibodies are sometimes clinically significant for transfusion and sometimes insignificant. Only three cases of serious HDFN due to anti-Ge3 have been reported.
- ✓ The Cromer antigens are carried on the decay accelerating factor and are distributed in body fluids and on RBCs, WBCs, platelets, and placental tissue.
- ✓ The rare anti-Cr^{a} and anti-Tc^{a} have been found only in black individuals; some examples have caused HTRs.
- ✓ The Knops antigens are located on complement receptor 1 (CR1). Knops antibodies are clinically insignificant and have weak and "nebulous" reactivity at the antiglobulin phase; they are not inhibited by plasma.
- ✓ The In^{a} antigen is more prevalent in Arab and Iranian populations, with In^{a} and In^{b} antigen expression being depressed on the dominant type Lu(a–b–) RBCs.
- ✓ JMH antibodies most often occur in individuals with acquired JMH– status. Anti-JMH in these individuals is not clinically significant.
- ✓ Anti-Vel is most often IgG but can be IgM, and has caused severe immediate HTRs and HDFN. When serum is tested, anti-Vel characteristically causes in vitro hemolysis.
- ✓ Anti-At^{a} has been found only in blacks; the antibody is usually IgG and has caused severe HTRs.
- ✓ Anti-Jr^{a} is found more commonly in Japanese, but clinical significance is not well established, since it is a rare antibody; it has caused HTRs and a fatal case of HDFN.
- ✓ Anti-Sd^{a} has characteristic shiny and refractile agglutinates under the microscope and is inhibited with urine from Sd(a+) individuals.

Review Questions

1. The following phenotypes are written incorrectly except for:

a. Jk^{a}+
b. Jka+
c. Jk^{a}(+)
d. Jk(a+)

2. Which of the following characteristics best describes Lewis antibodies?

a. IgM, naturally occurring, cause HDFN
b. IgM, naturally occurring, do not cause HDFN
c. IgG, in vitro hemolysis, cause hemolytic transfusion reactions
d. IgG, in vitro hemolysis, do not cause hemolytic transfusion reactions

3. The *Le* gene codes for a specific glycosyltransferase that transfers a fucose to the N-acetylglucosamine on:

a. Type 1 precursor chain.
b. Type 2 precursor chain.
c. Types 1 and 2 precursor chain.
d. Either type 1 or type 2 in any one individual but not both.

4. What substances would be found in the saliva of a group B secretor who also has *Lele* genes?

a. H, Le^{a}
b. H, B, Le^{a}
c. H, B, Le^{a}, Le^{b}
d. H, B, Le^{b}

5. Transformation to Le^{b} phenotype after birth may be as follows:

a. Le(a–b–) to Le(a+b–) to Le(a+b+) to Le(a–b+)
b. Le(a+b–) to Le(a–b–) to Le(a–b+) to Le(a+b+)
c. Le(a–b+) to Le(a+b–) to Le(a+b+) to Le(a–b–)
d. Le(a+b+) to Le(a+b–) to Le(a–b–) to Le(a–b+)

6. In what way do the Lewis antigens change during pregnancy?

a. Le^{a} antigen increases only
b. Le^{b} antigen increases only
c. Le^{a} and Le^{b} both increase
d. Le^{a} and Le^{b} both decrease

7. A type 1 chain has:

a. The terminal galactose in a 1-3 linkage to subterminal N-acetylglucosamine.
b. The terminal galactose in a 1-4 linkage to subterminal N-acetylglucosamine.
c. The terminal galactose in a 1-3 linkage to subterminal N-acetylgalactosamine.
d. The terminal galactose in a 1-4 linkage to subterminal N-acetylgalactosamine.

8. Which of the following best describes Lewis antigens?

a. The antigens are integral membrane glycolipids
b. Le^{a} and Le^{b} are antithetical antigens
c. The Le(a+b–) phenotype is found in secretors
d. None of the above